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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-------------|---|--|
| X — Y | WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6,10, 12, 13 and claims 1 and 5. | 1-3, 8-11, 18 4, 5, 13-17, 29-32, 34, 35, 37 |
| X — Y | US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8. | 1-3, 8-11, 18 4, 5, 13-17, 29-32, 34, 35, 37 |
| X,P | US 6,287,571 <i>B1</i> (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1. | 1, 9, 18 |
| X — Y | US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26. | 1-3, 8, 9-11, 18 4,5,13-17, 29-32, 34, 35, 37 |
| Y | WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683. | 1-3, 9-11, 13-18 |

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier application or patent published on or after the international filing date | "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

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C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| Y | NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods. | 1, 9, 29-32 |
| Y | PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract. | 1, 9, 29-32 |
| Y | LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. | 1, 9 |
| Y | PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract. | 16 |
| Y | NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract. | 1, 9 |

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

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BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

| Group | Claims | |
|-------|--|--|
| 1 | 1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Gag protein (SEQ ID NO: 29)</u> inserted in the <u>parallel orientation of E1</u> . In addition the vector contains a promoter and a polyadenylation signal. |
| 2 | 6, 7, 36 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29). |
| 3 | 12, 33 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the <u>antiparallel orientation of E1</u> . |
| 4 | 19-23, 38-42 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein. |
| 5 | 24, 27, 28, 43, 46, 47 | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle. |
| 6 | 25, 26, 44, 45 | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in <u>addition to administering a DNA plasmid vaccine</u> . |
| 7 | 48-51, 53, 54, 56 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the parallel orientation of E1. |
| 8 | 48-51, 53, 54, 56 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the parallel orientation of E1. |
| 9 | 48-51, 53, 54, 56 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the parallel orientation of E1. |
| 10 | 52 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the <u>antiparallel orientation of E1</u> . |
| 11 | 52 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in the <u>antiparallel orientation of E1</u> . |
| 12 | 52 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in the <u>antiparallel orientation of E1</u> . |
| 13 | 55 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> |

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| | | and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in <u>E1</u> . |
| 14 | 55 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in <u>E1</u> . |
| 15 | 55 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in <u>E1</u> . |
| 16 | 57-61 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an <u>HIV Pol protein</u> . |
| 17 | 62, 65, 66 | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle. |
| 18 | 63, 64 | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle <u>in addition to</u> administering a DNA plasmid vaccine. |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of <u>E1</u> . |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of <u>E1</u> . |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of <u>E1</u> . |
| 22 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of <u>E1</u> . |
| 23 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of <u>E1</u> . |
| 24 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of <u>E1</u> . |
| 25 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the antiparallel orientation of <u>E1</u> . |
| 26 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the antiparallel orientation of <u>E1</u> . |
| 27 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in <u>E1</u> . |
| 28 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in <u>E1</u> . |
| 29 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type |

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| | | adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in E1. |
| 30 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1 and ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in E1. |
| 31 | 76-80 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a <u>gene encoding an HIV Nef protein</u> . |
| 32 | 81, 84, 85 | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the <u>recombinant adenoviral particle</u> . |
| 33 | 82, 83 | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle <u>in addition to administering a DNA plasmid vaccine</u> . |
| 34 | 86a | The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from <u>three individual vectors</u> . |
| 35 | 86b, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from <u>one individual vectors</u> . |
| 36 | 86c, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from <u>two individual vectors</u> , one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> . |
| 37 | 86d, 87, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from <u>two individual vectors</u> , one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> . |
| 38 | 86e, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from <u>two individual vectors</u> , one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> . |
| 39 | 86f, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from <u>a single vectors as a fusion protein</u> . |
| 40 | 86g, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from <u>two individual vectors</u> . |
| 41 | 86h, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed <u>individually from one vector</u> . |
| 42 | 86i, 88 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from <u>two individual vectors</u> . |
| 43 | 86j, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from <u>individually from one vector</u> . |
| 44 | 86k, 88 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed <u>individually from one vector</u> . |
| 45 | 86l, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed <u>individually from one vector</u> . |
| 46 | 86m, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a <u>fusion protein from one vector</u> . |
| 47 | 86n, 88 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a <u>fusion protein from one vector</u> . |
| 48 | 86o, 88 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a <u>fusion protein from one vector</u> . |

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION

ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING
CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S.
provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2
(serial number unassigned), filed September 15, 2000, March 27, 2001, and
September 7, 2001, respectively.

10

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not Applicable

REFERENCE TO MICROFICHE APPENDIX

15

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first
generation adenovirus vaccines found to exhibit enhanced growth properties and
20 greater cellular-mediated immunity as compared to other replication-deficient vectors.
The invention also relates to the associated first generation adenoviral vectors
described herein, which, through the incorporation of additional 5' adenovirus
sequence, enhance large scale production efficiency of the recombinant, replication-
defective adenovirus described herein. Another aspect of the instant invention is the
25 surprising discovery that the intron A portion of the human cytomegalovirus (hCMV)
promoter constitutes a region of instability in adenoviral vector constructs. Removal
of this region from adenoviral expression constructs results in greatly improved vector
stability. Therefore, improved vectors expressing a transgene under the control of an
intron A-deleted CMV promoter constitute a further aspect of this invention. These
30 adenoviral vectors are useful for generating recombinant adenovirus vaccines against
human immunodeficiency virus (HIV). In particular, the first generation adenovirus
vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-
1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide
pharmaceutical products, and biologically active modifications thereof. Host
35 administration of the recombinant, replication-deficient adenovirus vaccines described
herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNase H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5' LTR-*gag-pol-env*-LTR 3' organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The *gag* gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the *pol* gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The *pol* gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNase H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNase H (RNase, p15) activities.

The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

5 The *env* gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

10 The *tat* gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The *rev* gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus
15 to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes
20 while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where
25 the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus
30 (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to
35 day to day viral loads seen throughout the course of disease.

Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺ T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0
5 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including *env* or *gag*. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region
10 are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of
15 incorporated individual A (packaging) repeats; see, e.g., Gräble and Hearing, 1990 *J. Virol.* 64(5):2047-2056; Gräble and Hearing, 1992 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, *Nature* 327: 716-717) and Larder, et al., (1989, *Proc. Natl. Acad. Sci.* 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on *in vitro* activity and infectivity related to interaction
20 with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, *FEBS Lett.* 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results
25 in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, *J. Biol. Chem.* 268: 2113-2119) disclose several
30 mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, *J. Virol.* 69: 376-386) disclose single and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral
35 replication functions.

It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, *gag*, *pol* and *nef*. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

to *pol* modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not

5 limited to *nef* modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining

10 the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-terminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

15 The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Pol- and/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based

20 adenovirus vaccine of the present invention is a purified recombinant, replication-defective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution

25 procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of

30 the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication-defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the

35 5' region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine
5 vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced
10 growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more
15 important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have,
20 therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine-vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or
25 biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-
30 3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1
35 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises:

- 5 a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene
10 expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

- Other aspects of this invention include a host cell comprising said adenoviral
15 vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

- To this end, the present invention particularly relates to harvested
20 recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6[®] cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material
25 which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

- Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual
30 an adenovirus vaccine vector comprising:

- a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto,
35 base pairs 3511-3523 of a wildtype adenovirus sequence, and,

b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

5 In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection
10 mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response
15 upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

20 To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine
25 plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then
30 a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In
35 these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

5 The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not
10 limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a multivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen
15 with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of
20 such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

 The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be
25 ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25)
30 within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second
35 harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

5 It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

10 It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

15 It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between from about base pair
20 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising: (i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a
25 polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV
30 infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a
35 single modality regimen.

As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to — highly active antiretroviral therapy —.

"first generation" vectors are characterized as being replication-defective.

5 They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

10 "s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

15 "Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

25 "Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

35 "Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as
5 exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an inactivated version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to
10 the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the
15 E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct
20 also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

25 "pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

30 "pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

35 "pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdeIE1sp1A" or "MRKpdeIE1(Pac/pIX/pack450)" or "MRKpdeIE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IAPol and G2A,LLAA nef genes directly into.

"MRKpdeIE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdeIE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt)" is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the *Bgl*III site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the LA pol gene in the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

5 "MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

10 "pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns
15 and/or V1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as
20 "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

25 Figure 1 shows the original HIV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

30 Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone
35 in the generation of the novel vectors of the instant invention.

Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

5 Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

15 Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

20 Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

25 Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

35 Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

5 Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed
10 herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences
15 through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate
20 consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding
25 sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as
30 underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine174
35 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

5 Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

10 Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

15 Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

20 Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

25 Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

30 Figure 31 shows the intracellular γ IFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti- γ IFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γ IFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+ γ IFN+ and CD4+ γ IFN+, respectively.

35 Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IAPol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IAPol fusion frame.

5

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus *cis*-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained its correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately 4,850 base pairs of foreign DNA inserts

(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; *see*, Chroboczek *et al.*, 1992 *J. Virology* 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6[®] cell line transfected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually out-compete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res* 19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities *in vitro* when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice *in vivo* with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

Preferred transcription termination sequences present within the gene expression cassette are the bovine growth hormone terminator/polyadenylation signal (bGHpA) and the short synthetic polyA signal (SPA) of 50 nucleotides in length, defined as follows: AATAAAAGATCTTTATTTTCATTAGATCTGTGTGTTGGT-TTTTTGTGTG (SEQ ID NO:26).

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on consensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized *env* sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as

5 MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both

10 constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEQ ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S.

15 Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon

20 optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a

25 construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs

30 disclosed herein relate to open reading frames for cloning to the enhanced first generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact

35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfri isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5-based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration
5 increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or tri-modality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include
10 any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef
15 constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviral-containing shuttle plasmids used in the construction of an adenovirus vector, this
20 plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses
25 the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression
30 regulatory elements, and a minimal pUC backbone; see Montgomery *et al.*, 1993, *DNA Cell Biol.* 12:777-783. The pUC sequence permits high levels of plasmid production in *E. coli* and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can
35 be used. Those of skill in the art will recognize that alternative vaccine plasmid

vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon
5 optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of
10 interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 *pol* open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine,
15 especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a
20 human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and
25 essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or
30 biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S.
35 Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost
5 administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at
10 least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine.
15 Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HIV-1 viral antigens, such as the
20 MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and
25 inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in
30 International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to
35 support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+).

Potential "2+1" divalent vaccines of the present invention might be a hCMV-gag-bGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with
5 hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (*e.g.*, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral
10 composition comprising an additional HIV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficacious adenovirus-based HIV-1 vaccine may be administered via a
15 combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

20 Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon.
25 Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino
30 acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of *E. coli* most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most
35 commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms--a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" *Advances in Pharmacology* 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed *supra*, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors
5 not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol,
10 pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were
15 harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby
20 incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®],
25 from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 *J. Gen. Virol* 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

30 It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be
35 used as human anti-HIV vaccines, and are capable of inducing immune responses.

Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as
5 buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM $MgCl_2$; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably
10 about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM $MgCl_2$, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface.
15 It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene
20 product. In general, an immunologically or prophylactically effective dose of 1×10^7 to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also
25 contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine
30 compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile
35 saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephalitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter

GMP grade pVIJnsHIVgag was used as the starting material to amplify the hCMV promoter. PVIJnsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery *et al.*, *supra* for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the *MscI* site of the hCMV promoter and a 3' primer (designed to contain the *BglIII* recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity *Taq* polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with *MscI* and *BglIII*. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following *MscI* and *BglIII* digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using *BglIII* digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the *BglIII* site. Colonies were screened using *SmaI* restriction enzymes to identify clones that carried the FLgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

AATAAAAGATCTTTATTTTCATTAGATCTGTGTG TTGGTTTTTTGTGTG
(SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: *In vitro* DNA transfection of original and new plasmid HIV-1 gag constructs.

| Plasmid | $\mu\text{g gag}/10\text{e}6 \text{ COS cells}/5\mu\text{g DNA}/48 \text{ hr}$ |
|--------------------------------------|--|
| HIVFL-gagPR9901 ^a | 10.8 |
| PVIns-hCMV-FLgag-bGHpA ^b | 16.6 |
| pV1Ins-hCMV-FLgag-SPA ^{b,c} | 12.0 |

^a GMP grade pV1Ins-hCMVintronA-FLgag-bGHpA.

5 ^b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

10

EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes

A rodent study was performed on the two new plasmid constructs described above – pV1InsCMV(no intron)-FLgag-bGHpA and pV1InsCMV(no intron)-FLgag-SPA - in order to compare them with the construct described above
 15 possessing the intron A portion of the CMV promoter, pV1InsHIVgag. Gag antibody and Elispot responses (described in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
 20 are hereby incorporated by reference) were measured. The results displayed in Table 3 below, show that the new plasmid constructs behaved equivalently to the original construct in Balb/c mice with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested, 20 μg and 200 μg .

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

| DNA ^a Promoter/terminator | Dose, ug ^b | Anti-p24 Titers (3 Wk PD1) ^c | | | SFC/10 ⁶ Cells (4 Wk PD1) ^d | | |
|---|--------------------------|--|------|------|--|------------|--------|
| | | GMT | +SE | -SE | Media | gag197-205 | p24 |
| HIVFL-gagPR9901 (GMP grade) | 200 | 12800 | 4652 | 3412 | 2(2) | 129(19) | 30(11) |
| | 20 | 5572 | 1574 | 1227 | 0 | 56(9) | 25(6) |
| pV1Jns-hCMV- FL-gag-bGHpA | 200 | 11143 | 2831 | 2257 | 0 | 98(5) | 12(6) |
| | 20 | 7352 | 2808 | 2032 | 0 | 73(9) | 11(6) |
| pV1Jns-hCMV- FL-gag-SPA | 200 | 16890 | 5815 | 4326 | 1(1) | 94(4) | 26(7) |
| | 20 | 5971 | 5361 | 2825 | 0 | 85(17) | 38(10) |
| Naïve | 0 | 123 | 50 | 36 | 0 | 0 | 0 |

^ain PBS^bi.m. Injections into both quads, 50 µL per quad^cn=10; GMT, geometric mean titer; SE, standard. error^dn=5, pooled spleens; mean of triplicate wells and standard. deviation. in parentheses;

Construction of the Modified Shuttle Vector -"MRKpdeIE1 Shuttle"

- The modifications to the original Ad5 shuttle vector (pdeIE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:
- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
 - (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).

These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdeIE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pAdHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdeIE1 shuttle) with *Pac*I and *Bst*Z1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either *Cla*I linearized pAdHVO (E3- adenovector) or *Cla*I linearized pAdHVE3 (E3+adenovector) into *E. coli* BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into *E. coli* XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple cloning site of the original shuttle vector contained *Cla*I, *Bam*HI, *Xho* I, *Eco*RV, *Hind*III, *Sal* I, and *Bgl* II sites. This MCS was replaced with a new MCS containing *Not* I, *Cla* I, *Eco*RV and *Asc* I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene.

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following co-infection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with *Hind*III (and *Pac*1 to remove the vector backbone) and then labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

5 Construction of the new shuttle vector containing modified gag transgene –
 “MRKpdeIE1-CMV(no intron)-FLgag-bGHpA”

 The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with *MscI* overnight and then digested with *SfiI* for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was
10 desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdeIE1 shuttle) was linearized by digestion with *EcoRV*, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated
15 together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdeIE1 shuttle vector.

20

EXAMPLE 9

Construction of the MRK FG Adenovectors

 The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdeIE1-CMV(no intron)-FLgag-bGHpA, was digested with *PacI*.
25 The reaction mixture was digested with *BsfZ171*. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with *ClaI* overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and ~100 ng of linearized MRKpAdHVE3 DNA were co-transformed into *E. coli* BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml
30 Terrific™ broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 µl dH₂O. A 2 µl aliquot of this DNA was transformed into *E. coli* XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml
35 LB +100 µg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme *BstEII* which cleaves

within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

EXAMPLE 11

Virus generation of an enhanced adenoviral construct – “MRK Ad5 HIV-1gag”

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested with *PacI* to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6[®] cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6[®] cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *HindIII* and radioactively labeled with [³³P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with *PacI/HindIII* prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (*in vitro* gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

particles each; 280 vp total) at passage 6 and continued to be passaged until P11.

Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral

5 material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture.

Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by
10 radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *HindIII* digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any
15 extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification
20 capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit
25 increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HIV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11
30 and 12 show no evidence of rearrangement.

Table 4:
Amplification Ratios Based on AEX and QPA Analysis of
Virus Amplification from Passage 3 to Passage 4.

| Ad gag construct | Amplification Ratio |
|------------------------|---------------------|
| MRKAd5gag | 470 |
| HCMV-Flgag-bGHpA [E3-] | 115 |
| HCMV-Flgag-SPA [E3+] | 320 |
| mCMV-FLgag-bGHpA [E3+] | 420 |
| Original construct * | 40 - 50 |

* This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

- 5 Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type
- 10 Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

| | Xv (10 ⁶ cells/ml), Infection | Viability (%) Harvest | Harvest Time h.p.i. | Cell Passage Number | Titer 10 ¹⁰ vp/ml culture | Titer 10 ⁶ vp/cell | QPA 10 ⁶ TCID ₅₀ /ml | Ratio AEX:QPA | Amplification Ratio 470 (MOI = 125) | AEX Internal Control |
|-----|---|--------------------------|------------------------|------------------------|---|----------------------------------|---|------------------|--|-------------------------|
| P4 | 1.49, 81% | 0.58, 50% | 44 | 46 | 8.7 | 5.9 | 1.72 | 50 | | |
| P5 | 1.38, 93% | 0.66, 47% | 48 | 49 | 6.7 | 4.9 | 1.38 | 49 | 170 | |
| P6 | 1.04, 94% | 0.68, 77% | 47 | 48 | 5.6 | 5.6 | 1.42 | 41 | 200 | |
| P7 | 1.50, 84% | 0.96, 61% | 49.5 | 50 | 3.9 | 1.4 | 0.87 | 40 | 50 | |
| P7 | 1.09, 97% | 0.78, 59% | 50 | 52 | 5.2 | 4.7 | 1.70 | 81 | 170 | |
| P8 | 1.03, 94% | 0.86, 64% | 47.5 | 54 | 8.0 | 6.7 | 1.10 | 82 | 310 | |
| P9 | 0.89, 95% | 0.99, 73% | 47.5 | 56 | 4.4 | 4.9 | 1.03 | 43 | 175 | 3.12 |
| P10 | 1.09, 91% | 1.06, 66% | 47.5 | 58 | 3.0 | 2.6 | 1.16 | 26 | 100 | 2.84 |
| P11 | 1.18, 88% | 0.88, 65% | 47 | 60 | 3.6 | 3.0 | 1.15 | 31 | 110 | 2.70 |
| P12 | 0.98, 91% | 0.85, 63% | 47.5 | 47 | 5.4 | 5.5 | 1.20 | 45 | 200 | 2.70 |
| P13 | 1.00, 88% | 0.70, 67% | 49 | 49 | 5.8 | 5.8 | 1.11 | 52 | 210 | 2.86 |
| P14 | 1.94, 92% | 0.88, 67% | 46 | 53 | 6.6 | 4.4 | | | 160 | 2.60 |
| P15 | 0.97, 96% | 0.64, 66% | 47 | 47 | 6.9 | 7.1 | | | 250 | 3.18 |
| | | | | | | | | | | 3.18 |
| | | | | | | | | | | 3.28 |
| | | | | | | | | | | 3.27 |
| | | | | | | | | | | 3.12 |
| | | | | | | | | | | 2.91 |

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

| | Xv (10 ⁶ cells/ml), Infection | Viability (%) Harvest | Harvest Time h.p.i. | Cell Passage Number | Titer 10 ¹⁰ vp/ml culture | Titer 10 ⁶ vp/cell | QPA 10 ⁶ TCID ₅₀ /ml | Ratio AEX:QPA | Amplification Ratio 300 (MOI = 125) | AEX Internal Control |
|-----|---|--------------------------|------------------------|------------------------|---|----------------------------------|---|------------------|--|-------------------------|
| P4 | 1.10, 97% | 1.28, 78% | 49 | 54 | 4.1 | 3.8 | 1.70 | 25 | | |
| P5 | 0.82, 89% | 1.18, 77% | 47 | 48 | 4.3 | 4.7 | 1.24 | 35 | 170 | |
| P6 | 1.55, 88% | 1.26, 76% | 49.5 | 50 | 1.2 | 0.8 | 0.56 | 21 | 30 | |
| P6 | 1.09, 97% | 1.11, 81% | 49 | 52 | 4.0 | 3.6 | 1.16 | 34 | 130 | |
| P7 | 1.17, 91% | 1.22, 91% | 47.5 | 54 | 3.7 | 3.2 | 0.50 | 74 | 110 | |
| P8 | 0.98, 88% | 1.41, 83% | 48 | 56 | 2.1 | 2.1 | 0.47 | 45 | 75 | 3.12 |
| P9 | 1.20, 89% | 1.26, 81% | 47.5 | 58 | 0.8 | 0.7 | 0.29 | 28 | 25 | 2.84 |
| P10 | 0.99, 82% | 1.65, 85% | 47 | 60 | 2.3 | 2.3 | 0.43 | 53 | 80 | 2.70 |
| P11 | 1.07, 96% | 1.25, 83% | 48 | 47 | 2.7 | 2.5 | 0.41 | 66 | 90 | 2.70 |
| P12 | 0.80, 91% | 1.14, 80% | 49.5 | 49 | 5.9 | 7.4 | 0.48 | 123 | 280 | 2.86 |
| P13 | 1.96, 95% | 1.14, 85% | 45.5 | 53 | 5.8 | 3.0 | | | 110 | 3.18 |
| P14 | 0.97, 96% | 1.03, 98% | 48.5 | 47 | 9.4 | 8.7 | | | 350 | 3.18 |
| P15 | 0.87, 99% | 0.97, 59% | 48.5 | 49 | 5.3 | 6.1 | | | 218 | 3.28 |
| | | | | | | | | | | 3.27 |
| | | | | | | | | | | 3.12 |
| | | | | | | | | | | 2.91 |
| | | | | | | | | | | 2.78 |
| | | | | | | | | | | 2.92 |

Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

5

MRKAd5gag(E3-)

| | Xv (10 ⁶ cells/ml), Viability (%) | | Harvest Time h.p.i. | Cell Passage Number | Titer 10 ¹⁰ vp/ml culture | Titer 10 ⁶ vp/cell | QPA 10 ⁶ TCID ₅₀ /ml | Ratio AEX:QPA | Amplification Ratio 100 (MOI=125) | AEX Internal Control |
|-----|--|-----------|------------------------|------------------------|---|----------------------------------|---|------------------|--|-------------------------|
| | Infection | Harvest | | | | | | | | |
| P4 | 1.62, 77% | 1.12, 62% | 47.5 | 46 | 2.0 | 1.2 | 0.92 | 20 | 100 | |
| P5 | 1.16, 92% | 0.62, 43% | 49 | 49 | 3.3 | 2.9 | 0.99 | 34 | 100 | |
| P6 | 1.71, 86% | 0.20, 10% | 49 | 50 | 4.7 | 2.7 | 1.70 | 28 | 100 | |
| P6 | 1.09, 97% | 0.63, 64% | 49.5 | 52 | 5.4 | 5.0 | 1.76 | 31 | 180 | |
| P7 | 1.17, 91% | 0.98, 72% | 47.50 | 54 | 7.1 | 6.1 | 0.67 | 106 | 220 | |
| P8 | 0.98, 88% | 0.77, 48% | 48 | 56 | 3.1 | 3.2 | 0.66 | 47 | 115 | 3.12 |
| P9 | 1.20, 89% | 1.03, 72% | 48 | 58 | 1.8 | 1.5 | 0.57 | 32 | 55 | 2.84 |
| P10 | 0.99, 82% | 0.60, 62% | 48.5 | 60 | 3.2 | 3.2 | 0.68 | 47 | 115 | 2.70 |
| P11 | 1.07, 96% | 0.88, 70% | 48.5 | 47 | 5.9 | 5.5 | 0.68 | 87 | 200 | 2.60 |
| P12 | 0.80, 91% | 0.67, 59% | 50 | 49 | 6.1 | 6.4 | 0.72 | 71 | 230 | 2.86 |
| P13 | 1.96, 95% | 0.91, 59% | 45.5 | 53 | 7.4 | 3.8 | | | 135 | 3.18 |
| P14 | 0.97, 96% | 0.81, 74% | 48 | 47 | 6.8 | 7.0 | | | 250 | 3.28 |
| P15 | 0.87, 99% | 0.84, 56% | 49 | 49 | 4.8 | 5.5 | | | 196 | 3.27 |
| | | | | | | | | | | 2.78 |
| | | | | | | | | | | 2.52 |

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (10^7 and 10^9 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: *In vitro* analysis for gag expression in COS cells by Elisa assay.

| Viral Vectors ^a | $\mu\text{g gag}/4.8 \times 10^5 \text{ COS}/10^8 \text{ parts}/48\text{hr}$ |
|----------------------------------|--|
| MRKAd5gag ^b | 1.40 |
| Clinical lot Ad5gag ^c | 1.28 |
| Research lot Ad5gag ^d | 1.32 |
| MCMVFL-gagbGHpA ^e | 0.42 |

^a $A_{260\text{nm}}$ absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

^d Research Ad5FLgag lot# 6399

^e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.

Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

| Group ID | Vaccine | Dose (vp) | GMT | SE upper | SE lower |
|----------|---|-----------------|--------|----------|----------|
| 1 | ^a MRKAd5gag | 10 ⁷ | 25600 | 5877 | 4780 |
| 2 | " | 10 ⁹ | 409600 | 94028 | 76473 |
| 3 | hCMV FL-gag bGHpA [E3-] → | 10 ⁷ | 7352 | 2077 | 1620 |
| 4 | " | 10 ⁹ | 235253 | 59767 | 47659 |
| 5 | hCMV FL-gag SPA [E3+] → | 10 ⁷ | 12800 | 9905 | 236 |
| 6 | " | 10 ⁹ | 310419 | 99181 | 75165 |
| 7 | ^b mCMV FL-gag bGHpA [E3+] → | 10 ⁷ | 44572 | 23504 | 15389 |
| 8 | " | 10 ⁹ | 941014 | 239068 | 190636 |
| 9 | ^c hCMV FL-gag bGHpA [E3-] ← | 10 ⁷ | 3676 | 934 | 745 |
| 10 | " | 10 ⁹ | 117627 | 17491 | 15227 |
| 11 | research lot hCMV intronA FL-gag bGHpA [E3-] <- | 10 ⁶ | 528 | 262 | 175 |
| 12 | " | 10 ⁷ | 14703 | 5274 | 3882 |
| 13 | " | 10 ⁸ | 58813 | 14942 | 11915 |
| 14 | " | 10 ⁹ | 204800 | 53232 | 42250 |
| 15 | clinical lot hCMVintronA FL-gag bGHpA [E3-] <- | 10 ⁶ | 230 | 82 | 61 |
| 16 | " | 10 ⁷ | 4222 | 3405 | 1138 |
| 17 | " | 10 ⁸ | 19401 | 3939 | 3274 |
| 18 | " | 10 ⁹ | 89144 | 25187 | 19639 |
| 19 | Naïve | none | 93 | 7 | 6 |

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro

Vaccination: T. Toner, Q. Su

Assay: M. Chen

^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The same lot of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) was used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10⁶ dose from this vector is 7 fold lower than the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

- 5 Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10¹¹ vp and 10⁹ vp. Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-
10 gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

- peripheral blood as summarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.
- 5

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with gag-expressing adenovectors (Protocol HIV203).

| Vaccine | Pre | Wk 4 | Wk 8 | Wk 12 | Wk 16 | Wk 20 | Wk 25 | Wk 28 |
|---|-----|------|------|-------|-------|-------|-------|-------|
| MRKAd5gag^a, 10¹¹ vp | | | | | | | | |
| 97N010 | <10 | 118 | 5528 | 11523 | 7062 | 21997 | ND | 51593 |
| 97N116 | <10 | 62 | 772 | 1447 | 1562 | 2174 | ND | 20029 |
| 98X007 | <10 | 66 | 3353 | 6156 | 6845 | 3719 | ND | 24031 |
| | | | | | | | | |
| MRKAd5gag, 10⁹ vp | | | | | | | | |
| 97N120 | <10 | 51 | 204 | 318 | 366 | 482 | ND | 6550 |
| 97N144 | <10 | 18 | 118 | 274 | 706 | 888 | ND | 7136 |
| 98X008 | <10 | 15 | 444 | 386 | 996 | 1072 | ND | 12851 |
| | | | | | | | | |
| Ad5gag^b, Clinical Lot, 10¹¹ vp | | | | | | | | |
| 97X001 | <10 | 87 | 2579 | 4718 | 7174 | 7250 | ND | 69226 |
| 97N146 | <10 | 72 | 3604 | 7380 | 7526 | 18906 | ND | 60283 |
| 98X009 | <10 | 78 | 4183 | 3946 | 3124 | 6956 | ND | 26226 |
| | | | | | | | | |
| Ad5gag, Clinical Lot, 10⁹ vp | | | | | | | | |
| 97N020 | <10 | <10 | 143 | 371 | 390 | 1821 | ND | 17177 |
| 97X003 | <10 | <10 | 39 | 93 | 156 | 596 | ND | 2053 |
| 98X012 | <10 | 81 | 342 | 717 | 956 | 1558 | ND | 11861 |
| ^a MRKAd5gag (hCMV, bGHpA, E3+) | | | | | | | | |
| ^b original Ad5gag vector (hCMV/intron A, bGHpA, E3-), lot#FN0001 | | | | | | | | |
| ND, not determined | | | | | | | | |

Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

| Grp # | Vaccination T=0,4,25 wks | Monkey ID | T=4 Wk | | T=6 Wk | | T=11 Wk | | T=16 Wk | | T=25 Wk | | T=28 Wk | |
|-------|--|--------------|--------------------|--------------------|--------|-------|---------|-------|---------|-------------------|---------|-------|---------|-------|
| | | | Media ^a | Gag H ^b | Media | Gag H | Media | Gag H | Media | Gag H | Media | Gag H | Media | Gag H |
| 1 | MRKAd5gag 10 ⁴ 11 vp | 97N010 | 6 | 89 | 0 | 395 | 0 | 1058 | 0 | 1174 | 3 | 775 | 4 | 1074 |
| | | 97N010(CD4-) | 4 | 38 | | | 3 | 993 | | | 0 | 76 | 0 | 594 |
| | | 97N116 | 1 | 398 | 1 | 609 | 0 | 534 | 4 | 395 | 1 | 261 | 0 | 408 |
| | | 97N116(CD4-) | 11 | 676 | | | 0 | 593 | | | 0 | 184 | 0 | 666 |
| | | 98X007 | 10 | 579 | 0 | 1304 | 3 | 2193 | 1 | 2118 | 3 | 1588 | 0 | 2113 |
| | | 98X007(CD4-) | 20 | 965 | | | 0 | 2675 | | | 0 | 1656 | 0 | 1278 |
| 2 | MRKAd5gag 10 ⁹ 9 vp | 97N120 | 5 | 275 | 1 | 249 | 4 | 141 | 4 | 119 | 9 | 206 | 4 | 219 |
| | | 97N120(CD4-) | 11 | 170 | | | 0 | 85 | | | 0 | 75 | 1 | 219 |
| | | 97N144 | 3 | 236 | 6 | 438 | 1 | 318 | 3 | 256 | 1 | 98 | 5 | 373 |
| | | 97N144(CD4-) | 6 | 148 | | | 0 | 285 | | | ND | ND | 0 | 625 |
| | | 98X008 | 4 | 368 | 1 | 1090 | 3 | 891 | 4 | 673 | 3 | 473 | 5 | 735 |
| | | 98X008(CD4-) | 14 | 696 | | | 0 | 1175 | | | 0 | 391 | 4 | 848 |
| 3 | Ad5gag clinical lot 10 ⁴ 11 vp | 97X001 | 0 | 281 | 1 | 485 | 0 | 817 | 0 | 1220 ^b | 1 | 894 | 0 | 1858 |
| | | 97X001(CD4-) | 10 | 283 | | | 3 | 996 | | | 0 | 1010 | 0 | 1123 |
| | | 97N146 | 3 | 150 | 1 | 465 | 0 | 339 | 1 | 1272 | 3 | 1238 | 3 | 1785 |
| | | 97N146(CD4-) | 6 | 133 | | | 0 | 370 | | | 0 | 654 | 0 | 971 |
| | | 98X009 | 0 | 93 | 3 | 339 | 3 | 559 | 0 | 896 | 1 | 384 | 0 | 1748 |
| | | 98X009(CD4-) | 0 | 73 | | | 0 | 333 | | | 0 | 225 | 0 | 644 |
| 4 | Ad5gag clinical lot 10 ⁹ 9 vp | 97N020 | 3 | 30 | 1 | 101 | 0 | 66 | 0 | 36 | 0 | 26 | 0 | 41 |
| | | 97N020(CD4-) | 10 | 29 | | | 0 | 15 | | | 0 | 1 | 0 | 16 |
| | | 97X003 | 4 | 68 | 5 | 134 | 0 | 18 | 1 | 38 | 4 | 38 | 6 | 81 |
| | | 97X003(CD4-) | 9 | 40 | | | 0 | 6 | | | 0 | 4 | 0 | 19 |
| | | 98X012 | 5 | 95 | 3 | 54 | 1 | 34 | 0 | 18 | 0 | 20 | 1 | 121 |
| | | 98X012(CD4-) | 11 | 70 | | | 0 | 11 | | | 0 | 8 | 0 | 41 |
| 5 | Naïve | 96R041 | 8 | 8 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| | | 053F | 14 | 18 | 5 | 16 | 20 | 14 | 19 | 15 | 10 | 15 | 24 | 9 |

Based on either 4x10⁶ or 2x10⁶ cells per well (depending on spot density)

ND, not determined

^aMock or no peptide control

^bPool of 20-aa peptides overlapping by 10 aa and encompassing the gag sequence

5

The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses *in vivo* even at a relatively low dose of 10⁹ vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

15

EXAMPLE 17

CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

20

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after
 5 review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wild-
 10 type (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize *in vivo* mammalian expression (Lathe, 1985, *J. Mol. Biol.* 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly
 15 (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It
 20 is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol
 25 protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprises codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized)") wherein DNA sequences encoding the protease
 30 (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:
 35 AGATCTACCA TGGCCCCCAT CTCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
 ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG

GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
 GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
 CACCCCCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGATGTGGG GGATGCCTAC
 5 TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
 AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
 CCTGACATTG TGATCTACCA GTACATGGAT GACCTGTATG TGGGCTCTGA CCTGGAGATT
 GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
 10 ACCCTTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
 CCCGACAAGT GGAAGTGTGA GCCCATTTGT CTGCCTGAGA AGGACTCCTG GACTGTGAAT
 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCTCCC AAATCTACCC TGGCATCAAG
 GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCC TGACTGAGGT GATCCCCCTG
 ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
 15 GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
 CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
 ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
 GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
 20 TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
 GGGGCTGAGA CCTTCTATGT GGATGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG
 AAGACTGAGC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
 GTGACTGACT CCCAGTATGC CCTGGGCATC ATCCAGGCC AGCCTGATCA GTCTGAGTCT
 25 GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
 GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGTCTGGC
 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCC AGGATGAGCA TGAGAAGTAC
 CACTCCAAT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG
 ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 30 TGCTCCCCTG GCATCTGGCA GCTGGACTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 GACAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGG GTCCATGAAC
 35 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCT CCACAACCTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC

GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 CAGATCACCA AGATCCAGAA CTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 5 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
 ID NO:1) .

The open reading frame of the wild type pol construct disclosed as SEQ ID
 NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg
 Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
 15 Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
 Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
 Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
 20 Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly
 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
 Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 25 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
 Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
 30 Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
 Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
 35 Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu
 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
 Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
 5 Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
 Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
 Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
 Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
 Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
 Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly
 Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu
 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 25 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which
 comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to
 deletion of the portion of the wild type sequence encoding the protease activity, a
 30 combination of active site residue mutations are introduced which are deleterious to
 HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present
 invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein
 the construct is devoid of DNA sequences encoding any PR activity, as well as
 containing a mutation(s) which at least partially, and preferably substantially,
 35 abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part
 and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

Table 1

| | <u>wt aa</u> | <u>aa residue</u> | <u>mutant aa</u> | <u>enzyme function</u> |
|----|--------------|-------------------|------------------|------------------------|
| | Asp | 112 | Ala | RT |
| | Asp | 187 | Ala | RT |
| 30 | Asp | 188 | Ala | RT |
| | Asp | 445 | Ala | RNase H |
| | Glu | 480 | Ala | RNase H |
| | Asp | 500 | Ala | RNase H |
| | Asp | 626 | Ala | IN |
| 35 | Asp | 678 | Ala | IN |
| | Glu | 714 | Ala | IN |

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

```

AGATCTACCA TGGCCCCCAT CTCCCCCAT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC
ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG
GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC
10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG
GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC
CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC
TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC
AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC
15 TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC
CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT
GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC
ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC
CCCGACAAGT GGAAGTGTGA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT
20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG
GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGAAGTGGT GATCCCCCTG
ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT
GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC
CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC
25 AGGATGAGGG GGGCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC
ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG
GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG
TTTGTGAACA CCCCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG
GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT
30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGAAGTACAC CACCAACCAG
AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT
GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT
GAGCTGGTGA ACCAGATCAT TGAGCAGCTG ATCAAGAAGG AGAAGGTGTA CCTGGCCTGG
GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC
35 ATCAGGAAGG TGCTGTTTCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC
CACTCCAACCT GGAGGGCTAT GGCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

```

ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGGAGG CCATGCATGG GCAGGTGGAC
 TGCTCCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
 GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
 GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGGC CTGTGAAGAC CATCCACACT
 5 GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
 AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
 AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
 GTGCAGATGG CTGTGTTTCAT CCACAAC TTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
 GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
 10 CAGATCACCA AGATCCAGAA CTTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCTGTGG
 AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
 GACATCAAGG TGGTGCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
 GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
 NO:3) .

15 In order to produce the IA-pol-based adenoviral vaccines of the present
 invention, inactivation of the enzymatic functions was achieved by replacing a total of
 nine active site residues from the enzyme subunits with alanine side-chains. As
 shown in Table 1, all residues that comprise the catalytic triad of the polymerase,
 namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues
 20 (Larder, et al., *Nature* 1987, 327: 716-717; Larder, et al., 1989, *Proc. Natl. Acad. Sci.*
 1989; 86: 4803-4807). Three additional mutations were introduced at Asp445,
 Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this
 IA Pol construct), with each residue being substituted for an Ala residue, respectively
 (Davies, et al., 1991, *Science* 252:, 88-95; Schatz, et al., 1989, *FEBS Lett.* 257: 311-
 25 314; Mizrahi, et al., 1990, *Nucl. Acids. Res.* 18: pp. 5359-5353). HIV pol integrase
 function was abolished through three mutations at Asp626, Asp678 and Glu714.
 Again, each of these residues has been substituted with an Ala residue (Wiskerchen,
 et al., 1995, *J. Virol.* 69: 376-386; Leavitt, et al., 1993, *J. Biol. Chem.* 268: 2113-
 2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene.
 30 The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and
 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro
 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys
 Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys
 35 Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala
 Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile
Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys
Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile
5 Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala
Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln
Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly
Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg
Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln
10 Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys
Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile
Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr
Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu
15 Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr
Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln
Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys
Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys
Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile
20 Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp
Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu
Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala
Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly
25 Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala
Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn
Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro
Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile
Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
30 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys
Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys
Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro
Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys
Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln
35 Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His
Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
 Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
 Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu
 5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
 Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
 Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
 Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro
 10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn
 Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
 Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations
 15 disclosed above may be suitable and therefore be utilized as an IA-pol-based
 adenoviral HIV vaccine of the present invention, either when administered alone or in
 a combined modality regime and/or a prime-boost regimen. For example, it may be
 possible to mutate only 2 of the 3 residues within the respective reverse transcriptase,
 RNase-H, and integrase coding regions while still abolishing these enzymatic
 20 activities. However, the IA-pol construct described above and disclosed as SEQ ID
 NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also
 preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1
 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal
 25 peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide
 such as is found in highly expressed mammalian proteins such as immunoglobulin
 leader peptides. Any functional leader peptide may be tested for efficacy. However,
 a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown
 herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein
 30 the pol coding region or a portion thereof is operatively linked to a leader peptide,
 preferably a leader peptide from human tPA. In other words, a codon optimized
 HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide
 at the amino terminal portion of the protein, which may effect cellular trafficking and
 hence, immunogenicity of the expressed protein within the host cell. As noted in
 35 Figure 16A-B, a DNA vector which may be utilized to practice the present invention
 may be modified by known recombinant DNA methodology to contain a leader signal

peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading

5 frame for the Pol protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized

10 herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity

15 is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted

20 and a human tPA leader sequence is fused to the 5' end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

25 GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
30 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCPTTAC
CATCCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
35 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG

GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATTT GTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA
 5 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGG CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC
 TGTGCAGAAG ATCACCACCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
 10 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGATGGG GCTGCCAACA GGGAGACCAA
 GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG AGCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 15 GGAGGTGAAC ATTGTGACTG ACTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 20 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGAC TGCACCCACC TGGAGGGCAA
 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGACAATG GCTCCAATT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 25 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCAGTCCC AGGGGGTGGT
 GGAGTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACCTCAGG GTGTACTACA GGGACTCCAG
 30 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA
 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:5).

The open reading frame of the wild type tPA-pol construct disclosed as SEQ
 35 ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:
 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 5 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr
 Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 15 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 30 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu
 5 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe
 Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6) .

The present invention also relates to a codon optimized HIV-1 Pol mutant
 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4)
 which comprises a leader peptide at the amino terminal portion of the protein, which
 may effect cellular trafficking and hence, immunogenicity of the expressed protein
 within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in
 the above paragraphs is suitable for fusion downstream of a leader peptide, such as a
 25 leader peptide including but not limited to the human tPA leader sequence. Therefore,
 any such leader peptide-based HIV-1 pol mutant construct may include but is not
 limited to a mutated DNA molecule which effectively alters the catalytic activity of
 the RT, RNase and/or IN region of the expressed protein, resulting in at least
 substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN
 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a
 leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the
 Pol coding region which effectively abolishes RT, RNase H and IN activity. An
 especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at
 least one point mutation which alters the active site and catalytic activity within the
 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially
 abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed
 5 herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open
 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
 CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCCTGTGAA
 15 GCTGAAGCCTT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
 CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
 CCCCAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
 GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
 GCTGGGCATC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT
 20 GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCCTTCAC
 CATCCCCCTC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
 GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCTCCATG ACCAAGATCC TGGAGCCCTT
 CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC
 TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG
 25 GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG
 CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC
 CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA
 CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGACCAAGG CCCTGACTGA
 GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA
 30 GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA
 GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC
 TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGA CTGAGGC
 TGTGCAGAAG ATCACCACCTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT
 GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT
 35 CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA
 GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGGCAAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA
 CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT
 GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA
 TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT
 5 GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT
 GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA
 GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT
 GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA
 TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA
 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC
 TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA
 GACCATCCAC ACTGCCAATG GCTCCAACCT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG
 GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT
 GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA
 15 CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT
 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA
 GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG
 GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT
 CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGACTA
 20 TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC
 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile
 Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val
 Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile
 Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu
 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr
 Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln
 Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys
 Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser
 Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro
 35 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu
 Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr
 Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln
 His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly
 Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp
 5 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val
 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val
 Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg
 Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile
 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile
 10 Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu
 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile
 Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met
 Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln
 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe
 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr
 Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro
 Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala
 Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly
 Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu
 20 Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala
 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr
 Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu
 Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu
 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp
 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile
 Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala
 Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val
 Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln
 Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu
 30 Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu
 Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu
 Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn
 Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala
 Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly
 35 Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val
 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly
 Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu
 Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp
 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly
 5 Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro
 Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly
 Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

10 CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed
 15 December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein
 20 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef
 25 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and
 30 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 35 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

5 As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

1. The nucleotide sequence of the codon optimized version of HIV-1 jfrl
10 nef gene is disclosed herein as SEQ ID NO:9, as shown herein:

GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCCGCG CCGACAGGGT GAGGAGGACC GAGCCCCGCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
15 GCTTCCCCGT GAGGCCCCAG GTGCCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCAGCATC AGGTTCCTCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC
20 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:9).

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG),
Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG);
25 Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG),
Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian
(human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby
incorporated by reference. See also Figure 19A-B for a comparison of wild type vs.
codon optimized nucleotides comprising the open reading frame of HIV-Nef.

30 The open reading frame for SEQ ID NO:9 above comprises an initiating
methionine-residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides
660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid
HIV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine
vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID
35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 5 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 10 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the
 15 inner surface of the host cell plasma membrane through myristylation of Gly-2
 (Franchini et al., 1986, *Virology* 155: 593-599). While not all possible Nef functions
 have been elucidated, it has become clear that correct trafficking of Nef to the inner
 plasma membrane promotes viral replication by altering the host intracellular
 environment to facilitate the early phase of the HIV-1 life cycle and by increasing the
 20 infectivity of progeny viral particles. In one aspect of the invention regarding
 codon-optimized, protein-modified polypeptides, the nef-encoding region of the
 adenovirus vector of the present invention is modified to contain a nucleotide
 sequence which encodes a heterologous leader peptide such that the amino terminal
 region of the expressed protein will contain the leader peptide. The diversity of
 25 function that typifies eukaryotic cells depends upon the structural differentiation of
 their membrane boundaries. To generate and maintain these structures, proteins must
 be transported from their site of synthesis in the endoplasmic reticulum to
 predetermined destinations throughout the cell. This requires that the trafficking
 proteins display sorting signals that are recognized by the molecular machinery
 30 responsible for route selection located at the access points to the main trafficking
 pathways. Sorting decisions for most proteins need to be made only once as they
 traverse their biosynthetic pathways since their final destination, the cellular location
 at which they perform their function, becomes their permanent residence.
 Maintenance of intracellular integrity depends in part on the selective sorting and
 35 accurate transport of proteins to their correct destinations. Defined sequence motifs
 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-
5 alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector
10 or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef
15 protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1
20 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down
25 regulation of CD4 (Aiken et al., 1994, *Cell* 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, *Nature Medicine* 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector
30 HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter
35 function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
 TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
 25 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
 CGTGCGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCAGGAG GACGAGGAGG TGGGCTTCCC
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
 30 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
 GCCCCAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCATGTC
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTTCG ACTCCAAGCT
 GGCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGTAC TACAAGGACT GCTAAAGCC
 35 (SEQ ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 10 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 15 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12).

Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human.

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jfrl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
 GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
 CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
 ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
 5 GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
 TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
 AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
 ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
 CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
 10 CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
 CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
 AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

15 Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val
 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg
 Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu
 Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp
 Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val
 20 Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp
 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His
 Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln
 Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg
 Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro
 25 Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His
 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu
 Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu
 His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA
 30 molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation
 site and dileucine motif have been deleted, as well as comprising a tPA leader peptide.
 This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which
 encodes a Nef protein containing a tPA leader sequence fused to amino acid residue
 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174
 35 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
 TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
 5 GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
 CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
 CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
 CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
 CTTCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
 10 CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
 CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGA
 GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCATGTC
 CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTTC ACTCCAAGCT
 GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCAGGTAC TACAAGGACT GCTAAAGCCC
 15 (SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly
 Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro
 20 Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala
 Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val
 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala
 Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu
 Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr
 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu
 Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp
 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro
 Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu
 Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn
 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu
 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His
 Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16).

An adenoviral vector of the present invention may comprise a DNA sequence,
 regardless of codon usage, which expresses a wild type or modified Nef protein as
 35 described herein, including but not limited to modified Nef proteins which comprise a
 deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175

and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdeIE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*III site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)ClaI (or MRKpAdHVE3) pre-plasmid. The vector, similar to the original shuttle vector contains the *Pac*I site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Jns-HIV-pol-inact(opt). Digestion of this plasmid with *Bgl* II releases the pol

gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the *Bgl*III site. The clones were checked for the correct orientation of the gene by using
 5 restriction enzymes *Dra*III/*Not*I. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FL-pol+bGHpA(S) was digested with restriction enzymes *Pac*I and *Bst*1107 I (or its
 10 isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*I digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)*Cla*I. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA
 15 sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

20 *Generation of research-grade recombinant adenovirus* - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAd5pol was digested with restriction enzyme *Pac*I (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate co-
 25 precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech Inc.). *Pac*I digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at ≤ -60°C. This pol containing
 30 recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

35 *Construction of vector: shuttle plasmid and pre-adenovirus plasmid* - Key steps performed in the construction of the vectors, including the pre-adenovirus

plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHPA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdelE1+CMVmin+BGHPA(str.) shuttle vector at the *Bgl*11 site. The clones were checked for correction orientation of the gene by using restriction enzyme *Sca*1. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHPA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHPA(s) was digested with restriction enzymes *Pac*1 and *Bst*1107 I (or its isoschizomer, *Bst*Z107 I) and then co-transformed into *E. coli* strain BJ5183 with linearized (*Cla*1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHPA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent *E. coli* XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 µg of pMRKAdnef was digested with restriction enzyme *Pac*1 (New England Biolabs) and 3.3 µg was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate co-precipitation technique (Cell Pfect Transfection Kit, Amersham Pharmacia Biotech

Inc.). *Pac1* digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6[®] cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at $\leq -60^{\circ}\text{C}$. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied by Frank Graham, McMaster University) using the primer set: mCMV (*Not* I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEQ ID NO: 20); mCMV (*Bgl* II) Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the *Not* I and the *Bgl* II sites respectively for each primer. This PCR amplicon was used for the construction of the mCMV shuttle vector containing the transgene in the E1 parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with *Not* I and *Bgl* II. The mCMV promoter (*Not* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (*Asc* I) Forward: 5'- ATA AGA ATG GCG CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (*Bgl* II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the *Asc* I and *Bgl* II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with *Asc* I and *Bgl* II to remove the hCMV-gag portion of the transgene. The mCMV promoter (*Asc* I/*Bgl* II digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with *Bgl* II and the gag reporter gene (*Bgl* II fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique

Bgl II site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Ins plasmids by *Bgl* II digestion.

EXAMPLE 22

5 Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac*I and *Bst*Z110I digestion of each shuttle vector was performed and each specific transgene
10 fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla*I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant pre-plasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently
15 prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

20 The tpa-nef gene was amplified out from GMP grade pV1Ins-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with *Bam*HI, gel purified and cloned into the *Bgl* II site of MRKAd5CMV-bGHpA shuttle vector (*Bgl* II digested and calf intestinal phosphatase treated).
25 Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following *Sca*I digestion. The resulting MRKAd5tpanef shuttle vector was digested with *Pac*I and *Bst*Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial
30 homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c
35 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁷ vp and 10⁹ vp; and (2) MRKAd5hCMV-IApol (E3-) at either

10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were collected from all the animals for RT ELISA and IFN γ ELISpot analyses, respectively. For all rodent immunizations, the Ad5 vectors were
5 diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 μ L aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following
10 vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and 10⁹ vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second dose, sera and spleens were
15 collected from all the animals for RT ELISA and IFN γ ELISpot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10⁹ vp and 10¹¹ vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either
20 10⁹ vp and 10¹¹ vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁹ vp and 10¹¹ vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0)
25 into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester, NY) were coated by overnight incubation with 100 μ L of 1 μ g/mL HIV-1 RT protein
30 (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 μ L of 1 μ g/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Huntsville, AL) and incubated for 2 h with 200 μ L/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was
35 performed followed by 4-fold serial dilution. 100- μ L aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO₄ per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELISpot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INF γ -secreting cells from mouse spleens (Miyahira, et al.1995, *J. Immunol. Methods* 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5×10^6 /mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, *Current Protocols in Immunology*. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 μ L/well of either 5 μ g/mL purified rat anti-mouse IFN- γ IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 μ g/mL mouse anti-human IFN- γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 μ L/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μ L of cell samples ($4-5 \times 10^5$ cells per well) and 50 μ L of the antigen solution were added. To the control well, 50 μ L of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 μ g/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺ T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HIV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 µL/well of either 1.25 µg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 µg/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 µL/well 1/2500 dilution of streptavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 µL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 µL of each sample is incubated with 15 µL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 µL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 µL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNγ ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10⁷ vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

| Group | Vaccine | Dose | No. of Doses | Anti-RT IgG Titers ^a | | | SFC/10 ⁶ cells ^b | | |
|-------|-----------------------|--------------------|--------------|---------------------------------|--------|--------|--|-------------------|-------------------|
| | | | | GMT | +SE | -SE | Medium | CD4+ peptide pool | CD8+ peptide pool |
| 1 | MRKAd5hCMVFLpol (E3+) | 10 ⁷ vp | 2 | 310419 | 301785 | 153020 | 1(1) | 75(4) | 2313(87) |
| | | | 1 | 919 | 372 | 265 | 1(1) | 72(9) | 533(41) |
| 2 | MRKAd5hCMVFLpol (E3+) | 10 ⁹ vp | 2 | 1838400 ^b | 0 | 0 | 2(2) | 114(9) | 2083(182) |
| | | | 1 | 713155 | 528520 | 303555 | 1(1) | 48(7) | 733(89) |
| 3 | MRKAd5hCMVFLpol (E3-) | 10 ⁷ vp | 2 | 310419 | 386218 | 172097 | 0(0) | 223(7) | 2807(27) |
| | | | 1 | 6400 | 14013 | 4393 | 10(8) | 141(21) | 409(28) |
| 4 | MRKAd5hCMVFLpol (E3-) | 10 ⁹ vp | 2 | 1838400 ^b | 0 | 0 | 1(1) | 160(13) | 2385(11) |
| | | | 1 | 1241675 ^b | 396725 | 300681 | 0(0) | 39(13) | 833(83) |
| 5 | Naïve | none | none | 57 | 9 | 7 | 9(2) | 11(4) | 10(1) |

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNear or at the upper limit of the serial dilution; hence, could be greater than this value^cNo. of Spot-forming Cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

- 5 C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10⁷ vp and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10⁷ vp and 10⁹ vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELISpot assay.
- 10

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

| Group | Vaccine | Dose | No. of Doses | Anti-nef IgG Titers ^a | | | SFC/10 ⁶ cells ^b | | |
|-------|-----------------------|--------------------|--------------|----------------------------------|-----|-----|--|--------------|---------------|
| | | | | GMT | +SE | -SE | Medium | aa51-70 CD8+ | aa81-100 CD4+ |
| 1 | MRKAd5hCMVFLnef (E3+) | 10 ⁷ vp | 2 | 174 | 70 | 50 | 1(1) | 1(1) | 1(1) |
| | | | 1 | 132 | 42 | 32 | 0(0) | 0(0) | 0(0) |
| 2 | MRKAd5hCMVFLnef (E3+) | 10 ⁹ vp | 2 | 174 | 70 | 50 | 0(0) | 61(7) | 4(2) |
| | | | 1 | 132 | 42 | 32 | 1(1) | 62(7) | 3(1) |
| 3 | MRKAd5mCMVFLnef (E3+) | 10 ⁷ vp | 2 | 132 | 42 | 32 | 3(1) | 15(5) | 5(2) |
| | | | 1 | 115 | 46 | 33 | 3(2) | 3(2) | 4(2) |
| 4 | MRKAd5mCMVFLnef (E3+) | 10 ⁹ vp | 2 | 132 | 42 | 32 | 4(2) | 83(13) | 5(1) |
| | | | 1 | 132 | 42 | 32 | 2(1) | 29(2) | 4(0) |
| 5 | MRKAd5mCMVtpanef(E3+) | 10 ⁷ vp | 2 | 132 | 42 | 32 | 3(2) | 14(2) | 5(1) |
| | | | 1 | 100 | 0 | 0 | 3(1) | 13(4) | 10(3) |
| 6 | MRKAd5mCMVtpanef(E3+) | 10 ⁹ vp | 2 | 230 | 170 | 98 | 3(2) | 145(29) | 4(0) |
| | | | 1 | 115 | 46 | 33 | 7(1) | 151(14) | 10(0) |
| 7 | Naïve | none | none | 152 | 78 | 52 | 21(2) | 18(6) | 28(3) |

^aGMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the geometric mean^bNo. of spot-forming cells per million splenocytes; mean values of triplicates are reported along with standard errors in parenthesis.

15

Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IAPol(E3+) and MRKAd5hCMV-IAPol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

- peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus Macaques.

| Vaccine (T=0,4 wks) | Monk # | Prebleed | | | T=4 | | | T=7 | | | T=16 | | |
|---------------------------------------|--------|----------|-------|-------|------|-------|-------|------|-------|-------|------|-------|-------|
| | | Mock | Pol L | Pol R | Mock | Pol L | Pol R | Mock | Pol L | Pol R | Mock | Pol L | Pol R |
| MRKAd5hCMV-IAPol(E3+) 10^{11} vp | 99C100 | 1 | 0 | 0 | 1 | 38 | 31 | 0 | 52 | 146 | 0 | 49 | 715 |
| | 99C215 | 1 | 2 | 2 | 10 | 98 | 249 | 1 | 109 | 305 | 22 | 88 | 250 |
| | 99D201 | 5 | 5 | 4 | 6 | 149 | 85 | 0 | 40 | 35 | 0 | 35 | 18 |
| MRKAd5hCMV-IAPol(E3+) 10^9 vp | 99D212 | 0 | 2 | 0 | 4 | 331 | 114 | 0 | 58 | 14 | 0 | 6 | 6 |
| | 99D180 | 0 | 4 | 2 | 0 | 19 | 182 | 4 | 38 | 158 | 5 | 38 | 108 |
| | 99C201 | 8 | 5 | 21 | 6 | 82 | 62 | 0 | 18 | 32 | 1 | 14 | 65 |
| MRKAd5hCMV-IAPol(E3-) 10^{11} vp | 99D239 | 5 | 2 | 2 | 20 | 82 | 172 | 1 | 68 | 114 | 9 | 21 | 40 |
| | 99C186 | 4 | 12 | 6 | 5 | 120 | 421 | 2 | 271 | 489 | 16 | 875 | 530 |
| | 99C084 | 1 | 8 | 9 | 8 | 84 | 464 | 0 | 14 | 238 | 1 | 24 | 264 |
| MRKAd5hCMV-IAPol(E3-) 10^9 vp | CC7C | 10 | 10 | 8 | 12 | 724 | 745 | 4 | 322 | 376 | 4 | 188 | 176 |
| | CD1G | 2 | 0 | 1 | 5 | 474 | 468 | 0 | 232 | 212 | 0 | 101 | 121 |
| | CD11 | 6 | 6 | 12 | 10 | 98 | 110 | 5 | 60 | 80 | 8 | 25 | 34 |
| Ndve | 083Q | nd | nd | nd | nd | nd | nd | 4 | 2 | 2 | 2 | 1 | 2 |

nd, not determined

Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

| RT ANTIBODY ASSAY TITERS IN mMU/mL | | | | |
|-------------------------------------|-----|------|------|------|
| Vaccine/Monkey Tag | T=4 | T=7 | T=12 | T=16 |
| MRKAd5hCMV-IAPol(E3+), 10^{11} vp | | | | |
| 99C100 | 61 | 1999 | 5928 | 4768 |
| 99C215 | 81 | 1541 | 2356 | 2767 |
| 99D201 | 53 | 336 | 539 | 387 |
| MRKAd5hCMV-IAPol(E3+), 10^9 vp | | | | |
| 99D212 | 10 | 40 | 49 | 68 |
| 99D180 | <10 | 36 | 79 | 93 |
| 99C201 | <10 | 37 | 71 | 76 |
| MRKAd5hCMV-IAPol(E3-), 10^{11} vp | | | | |
| 99D239 | 44 | 460 | 1234 | 1015 |
| 99C186 | 21 | 233 | 480 | 345 |
| 99C084 | 235 | 2637 | 2858 | 1626 |
| MRKAd5hCMV-IAPol(E3-), 10^9 vp | | | | |
| CC7C | 32 | 175 | 306 | 235 |
| CD1G | 20 | 140 | 273 | 419 |
| CD11 | 15 | 112 | 149 | 237 |

When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

10 Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

| Vaccine (T=0,4 wks) | Monk # | Pre | | T=4 | | T=7 | | T=16 | |
|---|--------|------|-----|------|------|------|------|------|------|
| | | Mock | Nef | Mock | Nef | Mock | Nef | Mock | Nef |
| MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ¹¹ vp | CD2D | 0 | 4 | 31 | 440 | 4 | 368 | 1 | 251 |
| | CC7B | 0 | 0 | 2 | 521 | 0 | 178 | 1 | 1522 |
| | CC61 | 2 | 9 | 31 | 112 | 0 | 108 | 11 | 100 |
| MRKAd5hCMV-nef(G2A,LLAA) (E3+) 10 ⁹ vp | CC2K | 9 | 9 | 6 | 52 | 0 | 35 | 0 | 15 |
| | CD15 | 5 | 4 | 30 | 998 | 2 | 586 | 0 | 434 |
| | CD16 | 6 | 1 | 6 | 1146 | 0 | 369 | 1 | 212 |
| MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ¹¹ vp | 99D191 | 1 | 5 | 4 | 614 | 0 | 298 | 2 | 419 |
| | 99D144 | 4 | 6 | 5 | 434 | 0 | 1100 | 2 | 932 |
| | 99C193 | 1 | 2 | 1 | 58 | 1 | 22 | 0 | 64 |
| MRKAd5mCMV-nef(G2A,LLAA) (E3+) 10 ⁹ vp | 99D224 | 1 | 11 | 14 | 231 | 1 | 125 | 0 | 70 |
| | 99D250 | 8 | 9 | 4 | 108 | 0 | 54 | 0 | 5 |
| | 99C120 | 1 | 6 | 20 | 299 | 0 | 92 | 0 | 79 |
| Naive | 083Q | nd | nd | 18 | 22 | 4 | 5 | 2 | 1 |

EXAMPLE 25

15 Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping by 10 amino acids. Four different peptide pools were tested for cross-clade recognition, and they were either derived from a clade B-based isolate (gag H-b; nef-
 20 b) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells from these patients presumably infected with clade B HIV-1 could recognize clade C gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated that these T cell responses against clade C gag peptide pool were about 60% of the clade B counterpart (Figure 24), while the T cell responses against clade C nef were
 25 about 85% of the clade B counterpart (Figure 25). These results suggest that cellular immune responses generated in patients infected with clade B HIV-1 can recognize gag and nef antigens derived from clade C HIV-1. These data show that a HIV vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapeutic advantage on a global scale.

5

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

| subject | bleed date | gag epitope # (from mapping) | mock | gag H-b | gagH-c | nef-b | nef-c |
|---------|------------|---------------------------------|------|---------|--------|-------|-------|
| #100 | 19-Jul-99 | 12 | 10 | 3950 | 1385 | 1295 | 1300 |
| #101 | 25-Jul-99 | 3 | 15 | 3885 | 1280 | na | 1020 |
| #102 | 25-Jul-99 | 4 | 15 | 1740 | 850 | 1255 | 1785 |
| #104 | 7-Jun-99 | 2 | 5 | 1355 | 1185 | na | 1060 |
| #107 | 11-Oct-99 | 2 | 25 | 3305 | 2795 | 670 | 870 |
| #405 | 11-Jul-99 | 2 | 15 | 4575 | 3180 | 1700 | 1500 |
| #501 | 19-Jul-99 | 2 | 15 | 1100 | 570 | 3365 | 3460 |
| #505 | 18-Jul-99 | 5 | 10 | 2145 | 1725 | 1235 | na |
| #506 | 28-Feb-99 | 2 | 25 | 150 | 45 | 400 | 610 |
| #701 | 28-Mar-99 | 5 | 30 | 7620 | 4775 | 3320 | 2780 |
| #709 | 17-May-99 | 3 | 15 | 2785 | 1945 | 1090 | 1630 |
| #710 | 24-May-99 | 4 | 5 | 1055 | 1080 | 2210 | 2140 |
| | | | | | | | |

10

EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

20

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

| Adenovector | AEX Titer (10 ¹⁰ vp/ml culture) | AEX Titer (10 ⁴ vp/cell) | Amplification Ratio |
|--------------------|---|--|------------------------|
| hCMV-FL-nef [E3+] | 1.1 | 0.9 | 30 |
| mCMV-FL-nef [E3+] | 2.2 | 2.1 | 75 |
| hCMV-tpa-nef [E3+] | 0.07 | 0.1 | 5 |
| mCMV-tpa-nef [E3+] | 1.3 | 0.9 | 35 |
| hCMV-FL-pol [E3+] | 2.7 | 2.1 | 75 |
| hCMV-FL-pol [E3-] | 1.9 | 1.3 | 45 |

- 5 *Roller Bottle Passaging* - Passaging of the *pol* and *nef* constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (triton-lysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by
10 restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

| | | Xviable (10 ⁶ cells/ml), Viability (%) | | Cell Passage Number | AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture | Titer 10 ⁴ vp/cell | Amplification Ratio | Triton Lysis Titer 10 ¹⁰ vp/ml culture |
|-------------------|------|---|-----------|---------------------|--|-------------------------------|---------------------|---|
| | | Infection | Harvest | | | | | |
| hCMV-FL-nef [E3+] | pool | 1.22, 85% | | 62 | 0.8 | 0.7 | 25 | 1.6 |
| | 1 | | 0.99, 62% | | | | | |
| | 2 | | 1.10, 72% | | | | | |
| hCMV-FL-pol [E3+] | pool | 1.42, 89% | | 62 | 4.5 | 3.2 | 115 | 7.0 |
| | 1 | | 1.22, 70% | | | | | |
| | 2 | | 1.42, 74% | | | | | |

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

| | | Xviable (10 ⁶ cells/ml), Viability (%) | | Cell Passage Number | AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture | Titer 10 ⁴ vp/cell | Amplification Ratio | Triton Lysis Titer 10 ¹⁰ vp/ml culture |
|-------------------|------|---|-----------|---------------------|--|-------------------------------|---------------------|---|
| | | Infection | Harvest | | | | | |
| hCMV-FL-nef [E3+] | Pool | 1.33, 90% | | 66 | 1.0 | 0.8 | 29 | 2.1 |
| | 1 | | 0.96, 70% | | | | | |
| | 2 | | 1.18, 73% | | | | | |
| hCMV-FL-pol [E3+] | Pool | 0.90*, 90% | | 56 | 4.2 | 4.7 | 168 | 6.5 |
| | 1 | | 1.18, 88% | | | | | |
| | 2 | | 1.04, 80% | | | | | |

- MRKAd5nef and MRKAd5pol Viral Production Kinetics* - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of
20 MRKAd5gag. PER.C6[®] cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral
25 particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6[®] cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

| | | Xv (10 ⁶ cells/ml), Viability (%) | | Cell Passage | AEX Titer | Titer | Amplification | Triton Lysis Titer |
|----------------------------|------|--|-----------|--------------|--------------------------------|-------------------------|---------------|--------------------------------|
| | | Infection | Harvest | Number | 10 ¹⁰ vp/ml culture | 10 ⁴ vp/cell | Ratio | 10 ¹⁰ vp/ml culture |
| hCMV-FL-nef (MRKAd5nef) | Pool | 1.11, 91% | | 60 | 1.5 | 1.4 | 50 | 2.8 |
| | 1 | | 1.23, 75% | | | | | |
| | 2 | | 1.34, 74% | | | | | |
| mCMV-FL-nef | Pool | 1.11, 91% | | 60 | 2.3 | 2.1 | 75 | 4.6 |
| | 1 | | 1.49, 84% | | | | | |
| | 2 | | 1.18, 77% | | | | | |

20

EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM L-glutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6[®] cells at a concentration of 0.2x10⁶ cells/ml. Cells were grown until they reached a cell concentration of approximately 1x10⁶ cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

- were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

| | |
|-------------|---------|
| Temperature | 36.5 °C |
| DO | 30% |
| PH | 7.30 |
| Agitation | 150 rpm |
| Sparging | None |

Table 21: Virus source used for experiments.

10

| Run | Batch ID | Cloned/Uncloned MRKAd5nef | MOI (vp/cells) |
|-----|-------------|---------------------------|----------------|
| #1 | B20010115-1 | Uncloned | 280 |
| | B20010115-2 | Uncloned | 280 |
| #2 | B20010202-1 | Cloned | 280 |
| | B20010202-2 | Cloned | 280 |

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

15

Table 22: Virus Concentration as measured by the AEX assay

| Run | Batch ID | Cloned/Uncloned MRKAd5nef | Virus Concentration @ 48hpi (1×10^{13} vp/L) | | | |
|-----|-------------|---------------------------|--|------------------|-------|---------------|
| | | | Supernatant | Clarified Lysate | Total | Triton Lysate |
| #1 | B20010115-1 | Uncloned | 0.72 | 3.26 | 3.98 | 5.76 |
| | B20010115-2 | Uncloned | 0.38 | 1.67 | 2.05 | 2.46 |
| #2 | B20010202-1 | Cloned | 0.80 | 6.00 | 6.80 | 8.88 |
| | B20010202-2 | Cloned | 0.50 | 6.00 | 6.50 | 8.47 |

Table 23: Virus Titers as measured by the QPA assay

| Run | Batch ID | Cloned/Uncloned MRKAd5nef | Virus Concentration @ 48hpi (1×10^{11} IU/L) | | | | |
|-----|-------------|---------------------------|--|-------------|------------------|-------|---------------|
| | | | Whole Broth | Supernatant | Clarified Lysate | Total | Triton Lysate |
| #1 | B20010115-1 | Uncloned | 0.13 | 1.12 | 1.76 | 2.88 | 11.28 |
| | B20010115-2 | Uncloned | 0.14 | 0.73 | 1.54 | 2.27 | 5.86 |
| #2 | B20010202-1 | Cloned | 0.14 | 0.97 | 1.62 | 2.69 | 11.89 |
| | B20010202-2 | Cloned | 0.14 | 1.17 | 1.70 | 2.97 | 12.47 |

20

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

5 MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pV1JnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of
10 V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10^7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10^7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

15 Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50
20 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

25 The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, $CD4^+$ -biased or $CD8^+$ -biased, and (b) boosting with the MRKAd5gag
30 construct produced in all cases a strongly $CD8^+$ -biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific $CD8^+$ T cells.

Table 24. Boosting of DNA/Adjuvant-Primed Rhesus Monkeys with MRKAd5gag
Number of SFC/million PBMCs

| Number of SF/Cmillion PBMCs | | Grp# | Priming T=0, 4, 8 wks DNA/5 mgs PBS (0101) | Boost T=28 wks MRKAd5gag(E3+) 10 ⁷ vp | Man# | T=0 | | T=4 | | T=6 | | T=10 | | T=17 | | T=24 | | T=28 | | T=30 | |
|-----------------------------|-------|------|--|---|--------------------------------------|------------------------|-------------------------|-----------------------|-----------------------------|--------------------------------|-----------------------|---------------------------------|------------------------|---------------------------------|-----------------------|--------------------------------|-------------------------|------------------------------------|-------------------------|-------------------------------------|-------|
| Medium | gag H | | | | | Medium | gag H | Medium | gag H | Medium | gag H | Medium | gag H | Medium | gag H | Medium | gag H | Medium | gag H | Medium | gag H |
| 1 | | | DNA/5 mgs + CRL 1005/45mgs | MRKAd5gag(E3+) 10 ⁷ vp | CB5H CC6X AW3G | NA 0 5 | NA 0 11 | 3 0 0 | 35 15 36 | 71 48 51 | 4 0 3 | 224 68 48 | 8 0 2 | 115 75 89 | 0 0 8 | 05 35 65 | 18 3 10 | 956 1705 989 | 0 1 0 | 316 755 395 | |
| 2 | | | DNA/5 mgs + CRL 1005/45mgs | MRKAd5gag(E3+) 10 ⁷ vp | CC1C CC1K AW3P CB5F AK8B | 0 4 9 NA 9 | 4 0 8 NA 12 | 1 1 1 0 4 | 60 101 10 31 36 | 111 284 71 288 119 | 0 0 4 0 1 | 270 791 164 530 439 | 4 5 8 19 0 | 280 492 104 374 425 | 8 0 6 9 0 | 232 321 85 251 316 | 3 0 11 8 4 | 959 1815 838 1549 1229 | 19 1 6 20 5 | 1345 1099 241 1734 1364 | |
| 3 | | | DNA/5 mgs + CRL 1005/7.5 mgs + 0.6 mM BAK | MRKAd5gag(E3+) 10 ⁷ vp | AW20 CA4R CB5B CB5W CB7D | 10 1 8 4 1 | 4 0 5 3 0 | 1 3 0 0 0 | 59 121 6 26 136 | 284 135 119 91 318 | 5 1 3 1 0 | 425 270 274 139 609 | 6 5 6 0 5 | 105 130 282 164 626 | 9 1 1 1 1 | 205 105 208 62 769 | 18 14 0 8 0 | 565 1384 838 643 2278 | 8 10 1 1 4 | 404 978 828 349 1831 | |
| 4 | | | none | None | 98D201 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 0 | 0 | 0 |

NA, not available

NA, not available

EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused
5 directly to the open reading frame of the IA pol gene (consisting of RT, RNaseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNase H and integrase (1350 amino acids; SEQ ID NO: 39).

10 The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR
15 product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

20 Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of
25 MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-
gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein
30 sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene
constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels
35 of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

5 **Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized HIV-1 gag, pol, gagpol, nef in rhesus macaques**

| Grp # | Vaccine T=0, 4 wks | Monk # | T=6 wks | | | | |
|-------|---|--------|---------|-------|---------|---------|------|
| | | | Mock | Gag H | Pol - 1 | Pol - 2 | Nef |
| 1 | MRKAd5 gag 10 ¹⁰ vp | CB9V | 0 | 15 | - | - | - |
| | | CD19 | 0 | 374 | - | - | - |
| | | 109H | 1 | 843 | - | - | - |
| 2 | MRKAd5 gag 10 ⁸ vp | 99D130 | 1 | 948 | - | - | - |
| | | W277 | 16 | 324 | - | - | - |
| | | 143H | 4 | 595 | - | - | - |
| 3 | MRKAd5 pol 10 ¹⁰ vp | CC1X | 4 | - | 46 | 256 | - |
| | | AW3W | 3 | - | 463 | 550 | - |
| | | AV43 | 6 | - | 95 | 1333 | - |
| 4 | MRKAd5 pol 10 ⁸ vp | AW38 | 1 | - | 19 | 30 | - |
| | | CC8K | 0 | - | 50 | 995 | - |
| | | CC21 | 1 | - | 33 | 436 | - |
| 5 | MRKAd5 nef 10 ¹⁰ vp | 076Q | 9 | - | - | - | 1204 |
| | | 091Q | 4 | - | - | - | 85 |
| | | 083Q | 0 | - | - | - | 176 |
| 6 | MRKAd5 nef 10 ⁸ vp | 00C029 | 1 | - | - | - | 114 |
| | | 98D022 | 6 | - | - | - | 170 |
| | | 98D160 | 3 | - | - | - | 198 |
| 7 | MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ¹⁰ vp each | 99D251 | 3 | 206 | 15 | 193 | 120 |
| | | 05H | 3 | 135 | 21 | 9 | 638 |
| | | 00C016 | 3 | 26 | 4 | 51 | 23 |
| 8 | MRKAd5gag+MRKAd5pol+MRKAd5nef 10 ⁸ vp each | 99D215 | 1 | 171 | 18 | 193 | 240 |
| | | 81H | 5 | 73 | 6 | 14 | 243 |
| | | 12H | 8 | 1140 | 115 | 811 | 719 |
| 9 | MRKAd5gagpol +MRKAd5 nef 10 ¹⁰ vp each | 99D211 | 0 | 83 | 56 | 838 | 725 |
| | | 22H | 4 | 385 | 119 | 1194 | 1915 |
| | | 61H | 4 | 343 | 11 | 765 | 853 |
| 10 | MRKAd5gagpol +MRKAd5 nef 10 ⁸ vp each | 34H | 3 | 78 | 19 | 5 | 75 |
| | | 48H | 1 | 65 | 105 | 46 | 43 |
| | | 70H | 5 | 158 | 15 | 220 | 191 |

Indicated are numbers of spot-forming cells per million PBMCs against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10⁶ PBMC.

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WHAT IS CLAIMED IS

:

1. A recombinant adenoviral vaccine vector at least partially deleted in
5 E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from
about base pair 1 to between from about base pair 400 to about
base pair 458 of a wildtype adenovirus genome; and
 - b) a gene encoding an HIV protein or immunologically relevant
10 modification thereof.
2. A vector in accordance with claim 1 comprising a packaging region
corresponding to from about base pair 1 to about base pair 450 of a wildtype
adenovirus genome.
3. A vector in accordance with claim 1 further comprising nucleotides
15 corresponding to between from about base pair 3511 to about 3524 to about base pair
5798 of a wildtype adenovirus genome.
4. A vector in accordance with claim 3 comprising base pairs
corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
5. A vector in accordance with claim 4 which is deleted of base pairs
20 451-3510.
6. A vector in accordance with claim 1 which is at least partially
deleted in E3.
7. A vector in accordance with claim 6 wherein the E3 deleted region
is from base pairs 28,133-30,818.

8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.

9. A vector in accordance with claim 1 wherein the vector comprises a
5 gene expression cassette comprising:

a) a nucleic acid encoding a protein;

b) a heterologous promoter operatively linked to the nucleic acid
encoding the protein; and

(c) a transcription termination sequence.

10 10. A vector in accordance with claim 9 wherein the gene expression
cassette is inserted into the E1 region.

11. An adenoviral vector in accordance with claim 9 wherein the gene
expression cassette is in an E1 parallel orientation

12. An adenoviral vector in accordance with claim 9 wherein the gene
15 expression cassette is in an E1 antiparallel orientation.

13. An adenoviral vector in accordance with claim 9 wherein the
promoter is a cytomegalovirus promoter devoid of intronic sequences.

14. An adenoviral vector in accordance with claim 13 wherein the
promoter is an immediate early human cytomegalovirus promoter.

20 15. An adenoviral vector in accordance with claim 9 wherein the
promoter is a murine cytomegalovirus promoter.

16. An adenoviral vector in accordance with claim 9 wherein the
transcription termination sequence is a bovine growth hormone polyadenylation and
transcription termination sequence.

17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

18. A cell comprising the adenoviral vector of claim 1.

19. Recombinant, replication-defective adenovirus particles harvested
5 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell line which expresses adenovirus E1 protein at complementing levels.

20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.

21. An HIV vaccine composition of claim 20 which comprises a
10 physiologically acceptable carrier.

22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant,
15 replication-defective adenovirus.

23. A method according to claim 22 wherein the cell is a PER.C6[®] cell.

24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
20 claim 21.

25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

27. A method according to claim 24 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.

29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.

30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.

31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

- i) SEQ ID NO: 29;
- ii) a heterologous promoter operatively linked to i); and
- iii) a transcription termination sequence.

32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.

33. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.

5 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

10 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.

37. A cell comprising the adenoviral vector of claim 30.

38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell
15 line which expresses adenovirus E1 protein at complementing levels.

39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.

40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.

20 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

42. A method according to claim 41 wherein the cell is a PER.C6[®] cell.

43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of
5 claim 21.

44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

10 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

15 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.

48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.

49. An adenoviral vector in accordance with claim 9 wherein the gene
20 expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.

50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of

SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.

52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.

53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.

56. A cell comprising the adenoviral vector of claim 49.

57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.

58. An HIV vaccine composition comprising purified adenovirus
5 particles of claim 57.

59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.

60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of
10 claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

61. A method according to claim 60 wherein the cell is a PER.C6[®] cell.

15 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.

63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with
20 a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.

5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.

68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.

10 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and

b) a gene expression cassette comprising

i) a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;

ii) a heterologous promoter operatively linked to i); and

iii) a transcription termination sequence.

70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.

72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.

5 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.

10 75. A cell comprising the adenoviral vector of claim 68.

76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.

15 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.

78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.

79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

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80. A method according to claim 79 wherein the cell is a PER.C6[®] cell.

81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.

82. A method according to claim 81 which further comprises
5 administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus
10 vaccine.

84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.

85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.

15 86. A multivalent adenovirus vaccine composition comprising recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a
20 gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:

- a) gag, pol, and nef, expressed independently from three individual vectors;

- b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- 5 c) gag, pol, and nef, expressed via two vectors, one expressing a pol-nef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gag-pol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nef-gag fusion and another expressing pol;
- 10 f) gag, pol, and nef, expressed via one vector expressing a gag-pol-nef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- 15 i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- 20 k) nef and gag, expressed independently from two individual vectors;
- l) nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

n) pol and nef, expressed via one vector expressing a pol-nef fusion;
and

o) nef and gag, expressed via one vector expressing a nef-gag fusion.

87. A multivalent adenovirus vaccine composition in accordance with
5 claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.

88. A multivalent adenovirus vaccine composition in accordance with
claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to distinct promoters and transcription termination sequences.

89. A multivalent adenovirus vaccine composition in accordance with
10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences
operatively linked to a single promoter; and the encoding nucleic acid sequences
operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

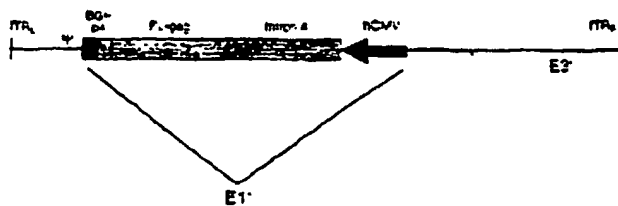


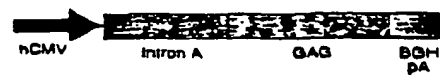
Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

[illegible]

Figure 2

Old Transgene:



New Transgenes:

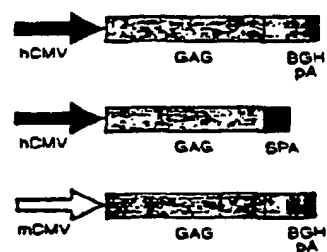


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

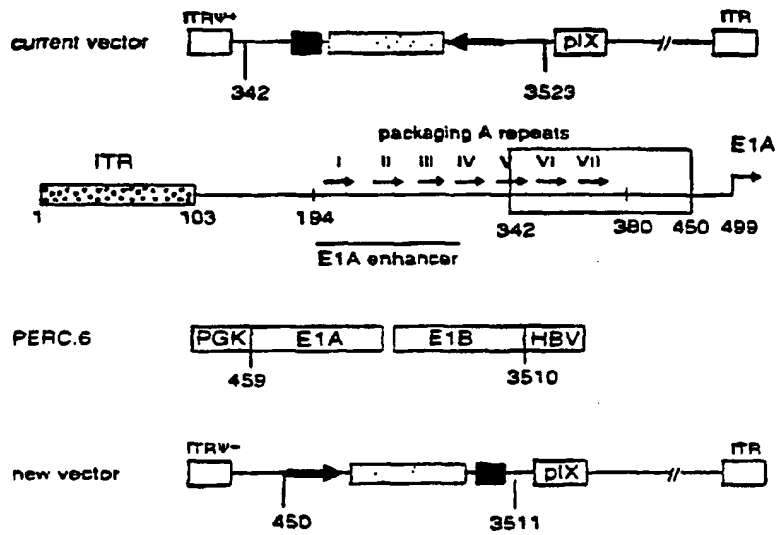


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

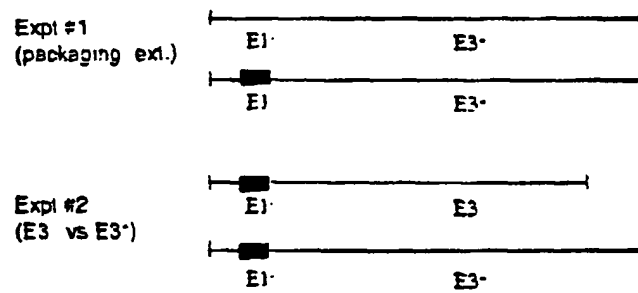


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.

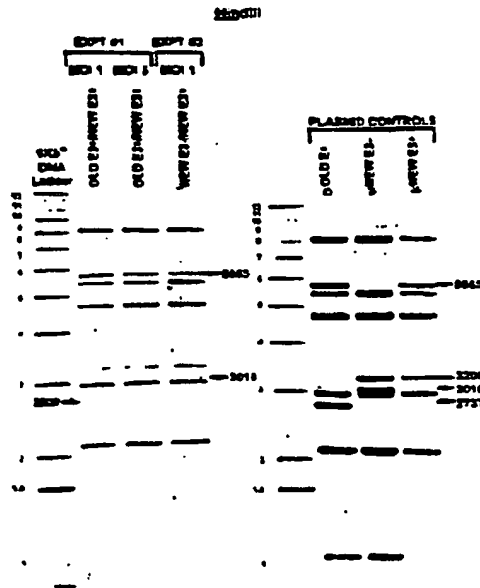


Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

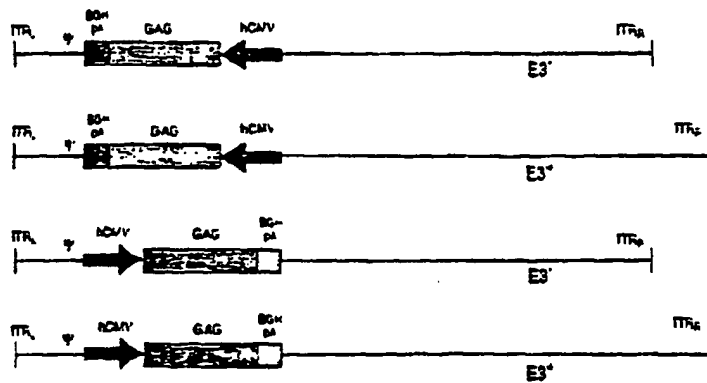


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

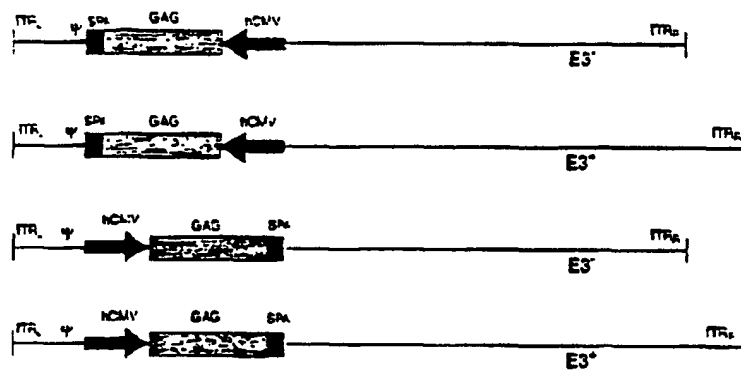


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

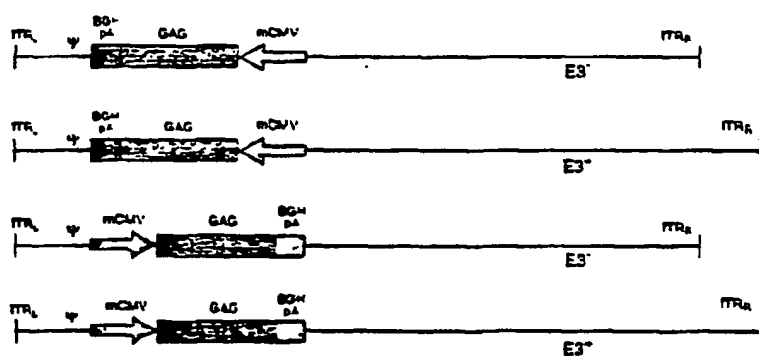


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

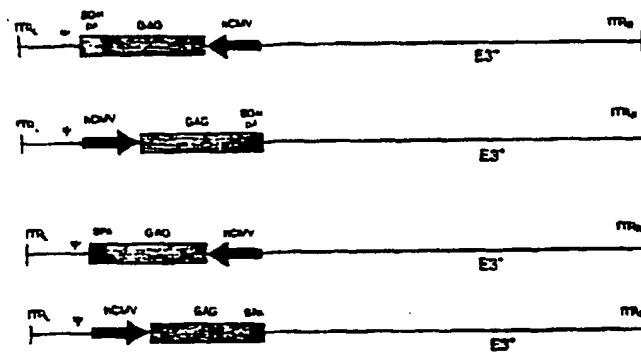


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

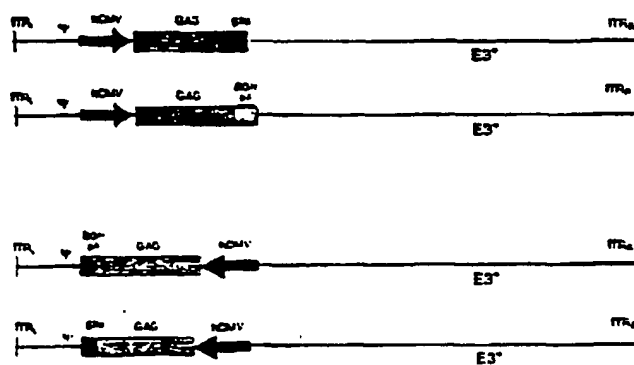


Figure 8B: Effect of polyadenylation signal

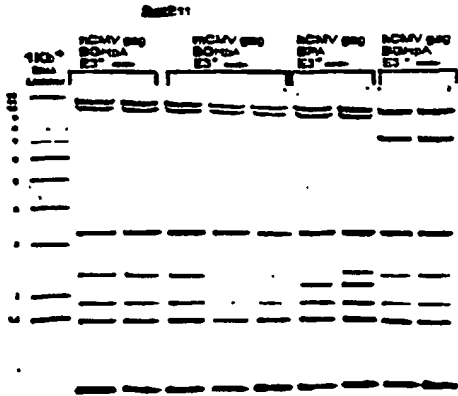


Figure 9: Viral DNA from the four Adgag candidates at P5, following BstE11 digestion.

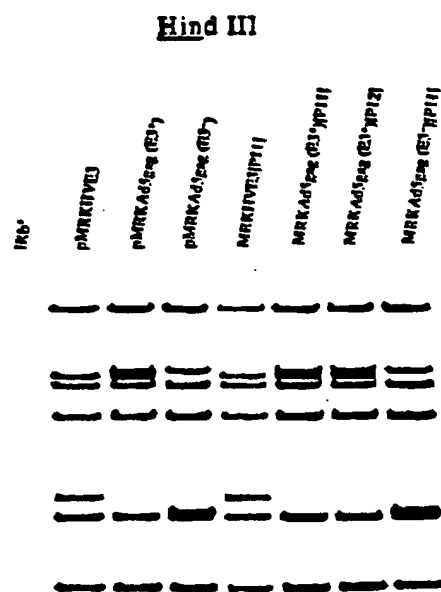


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

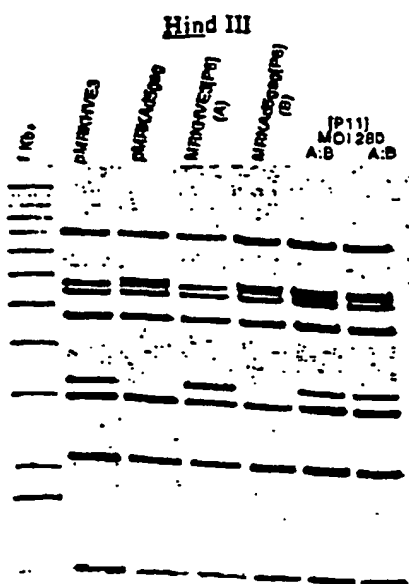


Figure 11: Viral DNA analysis (*Hind*III digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

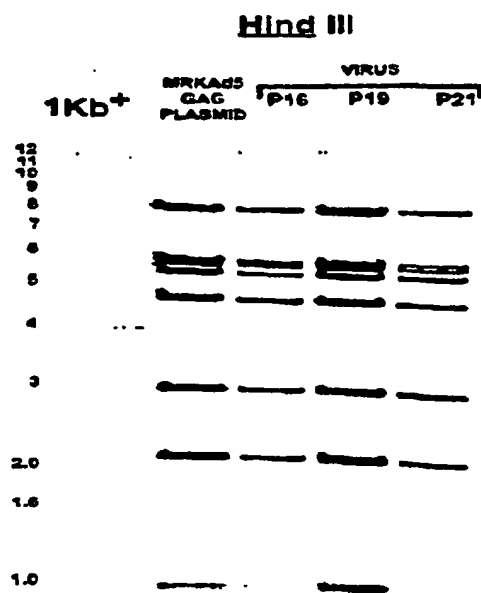
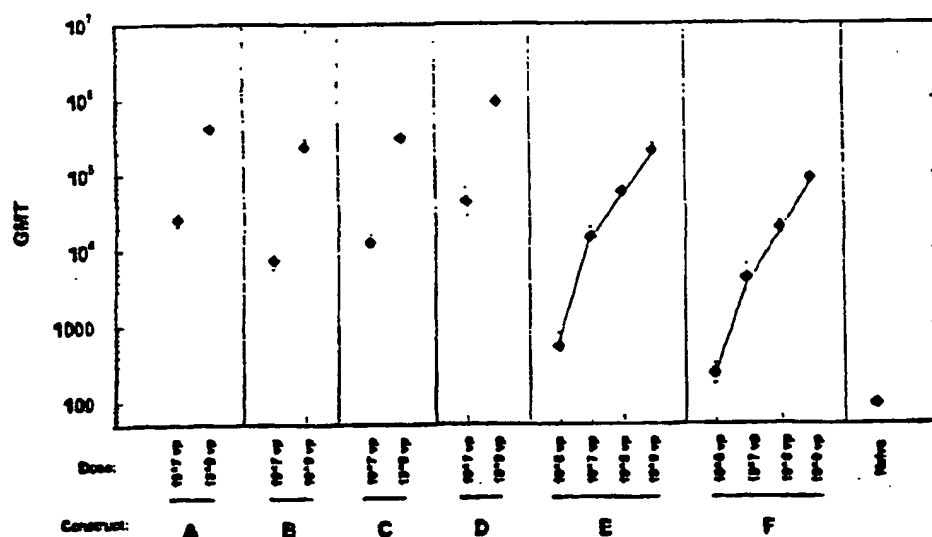


Figure 12: Viral DNA analysis by *Hind*III digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with *Pac*I and *Hind*III), and MRKAd5gag virus continually passaged to P16, P19 and P21 (serum containing media).

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Figure 1. Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb/c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5); (B) MRKAd5 E3⁺ bCMV-FLgag-bGHpA; (C) MRKAd5 E3⁺ bCMV-FLgag-SPA; (D) MRKAd5 E3⁺ mCMV-FLgag-bGHpA; (E) research lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reported are the geometric mean titers (GMT) for each cohort.



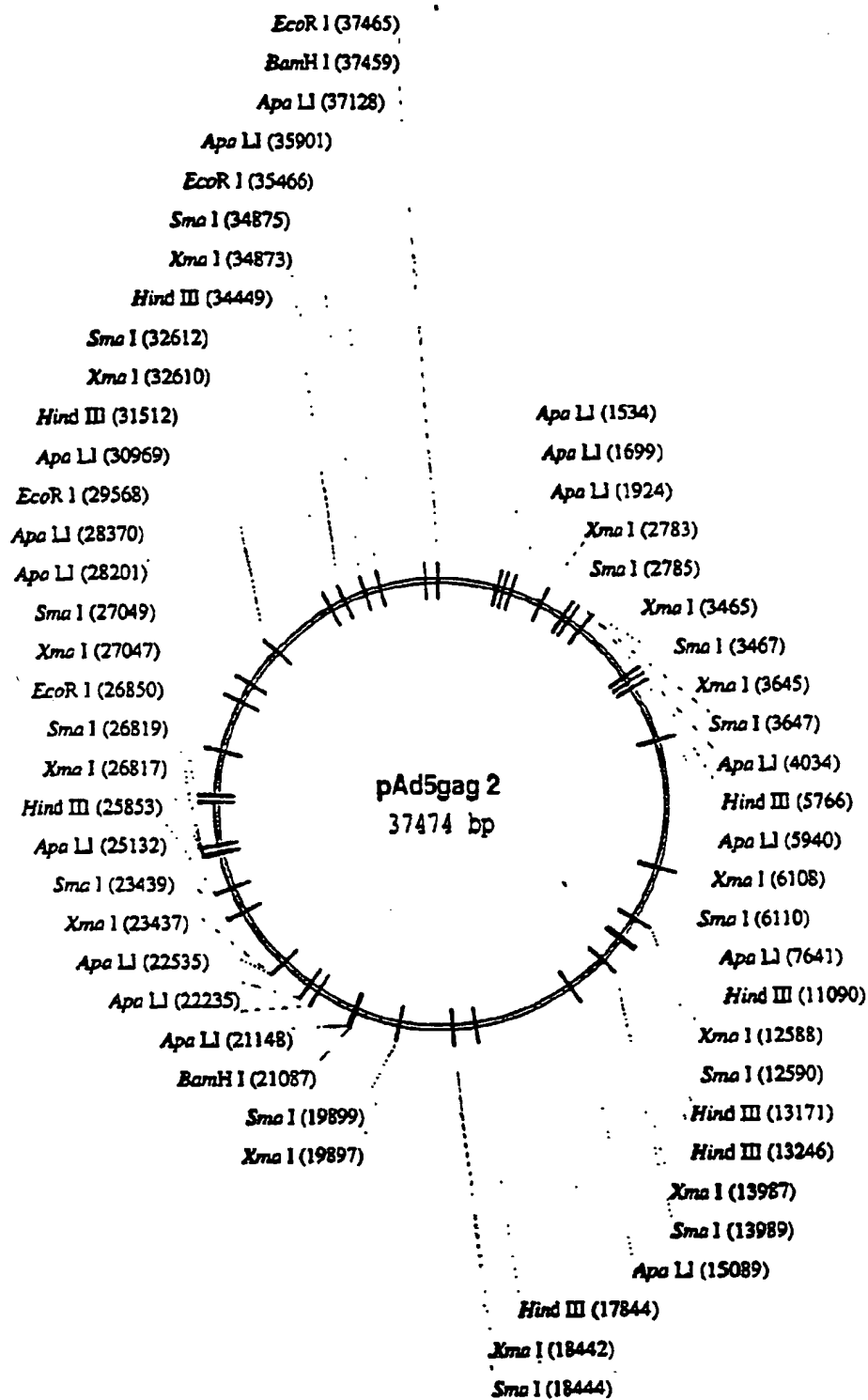


Figure 14

pMRRA15-9a4 MER682

1 TTTTAAATTA ACATCATCAA TAATATATCT TAATATATCT TAAATATCAAT ATGATATATCA GGTATATGAG AGGTATATCTT GGTATATCTT GGTATATCTT
 101 AAGATATTAAT TGTATATATTA ATATATATCTA ACTTATATCTA TACTATATCTA GGTATATCTT GGTATATCTT GGTATATCTT GGTATATCTT GGTATATCTT
 201 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 301 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 401 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 501 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 601 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 701 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 801 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 901 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1001 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1101 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1201 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1301 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1401 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1501 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA
 1601 GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA GGTATATCTA

Figure 1SA

pNRKAF15749 MER682

1701 CACGAGGCA TCTCCGCCG GACCTGTAT GCTGCTGTA AGTGTGTA GAGAGGTC TTCTCCCTG AGGTATCC CATTTCTCT GCTGTGTCG
 1801 GTGTCCGGT AGAGGGGCG CTGGAATTA CTGACCACT TTATGACT CTCTTCCG AGAGGGGAC TCCACTAGG ACCACTAGG AGTATCTT
 1901 TCCACCGTG GGGGTCTG GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2001 TGAATGGAC AGGTGATC GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2101 ACTACCGTG TCCGATGAT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2201 CAGGAGGCA TTGGCTGAT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2301 GTCTCTGCT AACGAGCTA CTGCTGTT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2401 ACTGCTGCT CTTGATGCT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2501 TGAAGGCTG TGAAGGCTG GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2601 GTCTCTGCT CTTGATGCT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2701 TGAAGGCTG TGAAGGCTG GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2801 GTCTCTGCT CTTGATGCT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 2901 TGAAGGCTG TGAAGGCTG GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 3001 GTCTCTGCT CTTGATGCT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 3101 TGAAGGCTG TGAAGGCTG GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG
 3201 GTCTCTGCT CTTGATGCT GAGTGTGTT AGATGATG TATGATG TATGATG TATGATG TATGATG TATGATG TATGATG

Figure 15B

pMRKAD5gag MER602

4901 GGTGCGCTTG AGGCTGATCC TCGTGTGCT GAAATTTTC GCGCTTTTC GCGCTGCTTC GCGCAGTTAG GATTGTACCA TGGTGTGATA GTCCAGCTCC
 5001 CCAGCGGAAAC TCCGACCAAG ACTACACAGA CTTCGCTAAC GTTCAGAACG GTACGCTCAG CCGCTCTCATC GTTAACTGGT ACCACAGTAT CAGGTCCGGT
 5101 TCCGCGCGGT GCGCTTTTCC GCGCACTTC GCGTATTTT ATGATTTTCA GATGATTTT TTTACAGTTT TTTACAGTTT TTTACAGTTT TTTACAGTTT
 5201 AGCGCGGCGG CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC CCGCTGCAAC
 5301 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 5401 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 5501 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 5601 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 5701 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 5801 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 5901 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 6001 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 6101 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 6201 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 6301 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC
 6401 TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC TCGTGTGCT GCGCTTTTCC

Figure 150

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| 6501 | GGTTCACGCA CGAAGGAGGC GTAGAGTCCG CGCAGCTTGT TTACTAGTTC GATCTGTGAC TTACGTTCTA GAGGACACTA GTCCAGGTTT TCTTGTATGA |
| 6601 | CGCAGTGGGT GCTTCTCTCG CATCTTCAGC GGTCTGACAA ACTGTTTGAG CCGCTACTCG AGCTGTGAT CCGCGGTAT CAGGTCCCAA AGCAACTACT |
| 6701 | TGTGATACTT ATCTGTGCCC TTTTTTTTCC AATGCTGAGG GTTGAATGAA AATCTTTCTG GTCTTTTCCA GTACTCTTGG ATCTGAAACC CTTGTGCTCT |
| 6801 | ACATATATGA TAGGACAGGG AAAAAAAGG TGTGAGTGC CAACTCTCTT TTGTAAGAGG CCGAATAGGT CAGTGAAGCC TAGGCTTTGG GCAAGCCGAA |
| 6901 | CGAAGGTGAA GAGCTTAGCA TGTGTAAGT GTTGAAGGAC GGTGAGGACC TTATAGGCTT TTCTACGGGT AGCGGTATG CCTGGCGGCG CTTTCCGAG |
| 7001 | AGGCTGCAAT CTGCGATCGT ACATCTTAGC CAACTGCGCG AATATCTGTT TGTATGGA AAGATGCTCA TCGGCGATAG TCGGCGCTT GAGGCGCTT |
| 7101 | GAGGTGTGGG TGAGCGCAAA GGTGTCTCTG ACTATGACTT TGATGATCTG GTATTGAG TCAGTGTGGT CCGATGCGCG GTGCTCCGAG AGCAAAAGT |
| 7201 | CTCCACACCC ACTGCGTTT CCACAGGGAC TGTACTGAA ACTTCATGAC CATTAATCTC AATTCACAGCA GCTTAAGCGG GATTAAGGTC TCGTTTCTCA |
| 7301 | CCGTGCGCTT TTTCGACGC GGAATTGACA GAGCGAAGGT GACATGTTT AMAGTATCT TTCTCTGAGG AATGCTGTTA TGCCTGAGGT TCGCTGAAAGT |
| 7401 | GACACGGAA AATCTTCCG CTTAAACCGT CCGGCTTCCA CTTGATGAA TTCTCATGTA AMRGGCGGC TCGGTATTTT TCGGTATTTT ACCECTTCTT |
| 7501 | TCCGCGACC TCGGACCGGT TGTAAATTAC CTGGCGGCG AGCACATCT CTTAAAGCC GTTGTATCTG TGGCCACAA TGTAAAGTTC CAGGAAGCGT |
| 7601 | AGGCGCGTGG AACTTGGCA ACATTTAATG GACCGCGCC TGGTCTTAG GCAATTTTCC GCACTACAGC TCGGCTTCTG TGGCTTCTG TGTCTTTCTG |
| 7701 | GGBATGCGCT TGAATGAGG CAATTTTATA AGTCTCTGTT TCAAGGACA TCCACTGAG AATGCGCTC GACTGGGCA CCGGCTACTA CTTCTACTT |
| 7801 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 7901 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 8001 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 8101 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 8201 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 8301 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 8401 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 8501 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 8601 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 8701 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 8801 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 8901 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 9001 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 9101 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 9201 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 9301 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 9401 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 9501 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 9601 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 9701 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |
| 9801 | CCATACGCTC TGTCTTACTC GAGGTGTCCA GTGCGCGTA ATCTGTAAGG TCCGACAGCG CTTTCTAGGA TAGTCTGAG CCGGCACTCA ATCTCGCGCT |
| 9901 | GGTGTGAGC GACGATGAG CTCACAGGT CACGCGCAT TAGTATTG TCAGATTGTC AGTGTGTCGC GAATGTTGCT AACTTGGCCA TTTTCTCTG |

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|------|--|------|--|------|---|---------|---|------|---|-------|---|-------|---|---------|---|-------|--|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|
| 9701 | ACAAAGCCGT GGTATCCGC CGTGTTCATG GTGTAAATTC AGTATGACAT AATGACATG TTAAAGGTCT GTTACCCCGT GTGTACCCCGT CTTGTAGAGC TCGTGTATC | KhoI | GGTATCCGC CGTGTTCATG GTGTAAATTC AGTATGACAT AATGACATG TTAAAGGTCT GTTACCCCGT GTTACCCCGT GTTACCCCGT GTTACCCCGT GTTACCCCGT | 9801 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | HindIII | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 9901 | GGGTACCCGT AGGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG | EcoRV | GGGTACCCGT AGGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG GGTTCCTCG | 10001 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | HindIII | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10101 | AAATGCTTAC GCTCTAGAC GTTCAAAAG GTTCAAAAG GTTCAAAAG GTTCAAAAG GTTCAAAAG GTTCAAAAG GTTCAAAAG GTTCAAAAG | 10201 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10301 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10401 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10501 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10601 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10701 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10801 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 10901 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 11001 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 11101 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC | 11201 | GTAAAGCCGC GAGTCAATA GTTATGCTGT GTATGCTTC ATGATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC GTTATGCTAC |
|------|--|------|--|------|---|---------|---|------|---|-------|---|-------|---|---------|---|-------|--|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|

Figure 15g

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|-------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 11301 | TGATTTGAT | AAACATCTG | CAGATGATG | TTTCTGATCA | CTTAAATTTG | ACTTATCTG | ACAACTGAT | CCCATCAAC | TATTCATGC | TTAGCTCTG |
| 11401 | AGCTAACTA | TTTGTAGGAC | GTCTCTATC | ACAACTCTT | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 11501 | CAAGTTTAC | GGCCGCAAGA | TATATCTAT | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 11601 | GGCTGCAAG | GGCCCTGCT | GGCTGCAAG | GGCTGCAAG | GGCTGCAAG | GGCTGCAAG | GGCTGCAAG | GGCTGCAAG | GGCTGCAAG | GGCTGCAAG |
| 11701 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 11801 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 11901 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12001 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12101 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12201 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12301 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12401 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12501 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12601 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12701 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |
| 12801 | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG | CTTAAATTTG |

Figure 15H

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|-------|---|---|---|---|-------|
| 12901 | GGATGATATG CCTCAACCG GCGTTTATC AACCGCTTA TTAATCTACTT GATATCTTG GATCGCTGA ACCCGATTA TTACACCAT GGTATCTTCA | CGGTACATAC GGAGTTTGCG CCGCAATAG TTAATTAAT ACCATATGA CTAATCTAT CCGCGCAT TTAATCTAT AAATGTTTA CTAATACAT | ACCCGACATG GCTACCGCC CCGTTTATC ACACCGTTC ATTCAGATG CTTAGATTA ACATATAT CTTCTCGAC GACATAGACG ACATCTGCT | TTGCGCTGAC CGATGCGCG GGACCAAGA TTAATCGCC TAAGCTAC TAATCTAT TGTACTTAA GAGAGCCCTG CTGTATCTGC TGTCCACAA | MinDB |
| 13001 | TTCCCGCAA CCGCAGACC TGTAGAGTT GTACAGATC GAGCAGGAG AGATGATG GTCAAGGAA AGCTTCCGA GTTGTCCGAT CTTGTCCGAT | AAGCGGCTT GCGCTCTGG AGATCTTCA CTTGTCTCG CTGTCTAT TCTTCGGA CTTCTCTT TCGAAGCGT CCGGTTCTGC GATACGCTT | TTCCCGCAA CCGCAGACC TGTAGAGTT GTACAGATC GAGCAGGAG AGATGATG GTCAAGGAA AGCTTCCGA GTTGTCCGAT CTTGTCCGAT | TTCCCGCAA CCGCAGACC TGTAGAGTT GTACAGATC GAGCAGGAG AGATGATG GTCAAGGAA AGCTTCCGA GTTGTCCGAT CTTGTCCGAT | MinDB |
| 13101 | CTAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG | CTAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG | CTAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG | CTAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG | MinDB |
| 13201 | AGAGAGATG CTTAAACAC TCCCTCTTC ATCGCGAGC CGAATAAC CTTCCTCG CATTCGCA CAACGGATA GAGAGCTAG TGGACATAT | TCTCTCTCAT GATTTCTTG AGCGAGAGC TCGAGCTGC GCTTTTTC GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG | CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG | MinDB |
| 13301 | CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG | CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG | CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG | CTATCTCA CTATCTCA GATGCTCT GAGCGCTG CCGCGCGCG CAGTCTAGA TCATCGCTA AGCTTCTGA CTATCTCA CTATCTCA GATGCTCT GAGCGCTG | MinDB |
| 13401 | GAGGAGATG ACTCGCAGA CCGAGGAGC GTCCTGAT TCGAGCTAG ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA | CTCTCTCTAC TCGAGCTAG CCGAGGAGC GTCCTGAT TCGAGCTAG ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA | CTCTCTCTAC TCGAGCTAG CCGAGGAGC GTCCTGAT TCGAGCTAG ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA | CTCTCTCTAC TCGAGCTAG CCGAGGAGC GTCCTGAT TCGAGCTAG ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA ACCCTCTC CAGAGCTTA | MinDB |
| 13501 | AAATAAATA GCATGATGA AAATAAATA CTCACCAAG CCAATCTAC GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | TTTTTTTTT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | TTTTTTTTT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | TTTTTTTTT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | MinDB |
| 13601 | TGAGGAGAT CTTCTCTCT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | TTTTTTTTT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | TTTTTTTTT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | TTTTTTTTT CTTACTTCTT TTTATTTTT GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG GATTCATG | MinDB |
| 13701 | ACTCTTCCA GAGAGAGGA GATCTCTC ACACCTCG CCGCGCGCG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG | ACTCTTCCA GAGAGAGGA GATCTCTC ACACCTCG CCGCGCGCG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG | ACTCTTCCA GAGAGAGGA GATCTCTC ACACCTCG CCGCGCGCG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG | ACTCTTCCA GAGAGAGGA GATCTCTC ACACCTCG CCGCGCGCG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG CAGAGCTAG | MinDB |
| 13801 | GTCTCTCTC GGTACTCTG CCGAGAGCA GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | GTCTCTCTC GGTACTCTG CCGAGAGCA GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | GTCTCTCTC GGTACTCTG CCGAGAGCA GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | GTCTCTCTC GGTACTCTG CCGAGAGCA GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | MinDB |
| 13901 | ACAGGTCAC GATGCTGCA TCCCTGAT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | ACAGGTCAC GATGCTGCA TCCCTGAT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | ACAGGTCAC GATGCTGCA TCCCTGAT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | ACAGGTCAC GATGCTGCA TCCCTGAT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | MinDB |
| 14001 | CACAGACAC ATCAATCTT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | CACAGACAC ATCAATCTT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | CACAGACAC ATCAATCTT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | CACAGACAC ATCAATCTT ACCGAGCA CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | MinDB |
| 14101 | GTGCTCTCG TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC | GTGCTCTCG TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC | GTGCTCTCG TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC | GTGCTCTCG TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC TGTGCTGAC | MinDB |
| 14201 | ACTACTCTCA GACCATGAC ATAGACTTA TCGAGAGC GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | ACTACTCTCA GACCATGAC ATAGACTTA TCGAGAGC GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | ACTACTCTCA GACCATGAC ATAGACTTA TCGAGAGC GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | ACTACTCTCA GACCATGAC ATAGACTTA TCGAGAGC GATCTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | MinDB |
| 14301 | TGATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | TGATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | TGATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | TGATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | MinDB |
| 14401 | GTATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | GTATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | GTATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | GTATGAGCT CTGTACTTG TATCTGAT ACTTCTTG CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT CTTACTTCTT | MinDB |

Figure 15I

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14501 CTTACAGTGA TCTGAGGCT GTTACATTC CCGACTTCT GTATGTGAC GTTACTACAG GTAGCTTGA AGATGACACC GAACAGGCG GAACAGGCG GGGGTGCTCT
 14601 GATCTACT AGACTTCCA CCATTGTAA GCGGTACAA CTTACATTC CTTAGACTT TCTACTGTC TCTACTGTC CTTTCTCCG CTTTCTCCG CTTTCTCCG
 14701 AGGCGCAC ACACAGCTG GCACTGTCG GATGAGTAC TCTACATTC TCTACATTC TCTACATTC TCTACATTC TCTACATTC TCTACATTC TCTACATTC
 14801 GCGGAGCT GTGACAGG GTGACAGG GTGACAGG GTGACAGG GTGACAGG GTGACAGG GTGACAGG GTGACAGG GTGACAGG GTGACAGG
 14901 CTTGCTAT ACCTAGCG ACCCTAGC ACCCTAGC ACCCTAGC ACCCTAGC ACCCTAGC ACCCTAGC ACCCTAGC ACCCTAGC ACCCTAGC
 15001 TTTGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA TATGAGCA
 15101 GCTGCTAT GATGAGCT GATGAGCT GATGAGCT GATGAGCT GATGAGCT GATGAGCT GATGAGCT GATGAGCT GATGAGCT GATGAGCT
 15201 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15301 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15401 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15501 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15601 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15701 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15801 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 15901 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT
 16001 GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT GCGGCTAT

Figure 15 J

[illegible]

Figure 15L

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|-------|--|-------|--|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|
| 21001 | TTATGTCAT GGGGCATC ACAGACTTG GCCAAACCT TCCTACTATC AACTGCTGC AGGCTATGA CATGACTTTT GAGTGTATC CCATGACGA | 21101 | ATATACAGTA CCGCGGTAG TGCTGTACC CGTCTTTGGA AGAGATGCTT TTGAGCTGAG ACCATGCTGA CCGCTGCTG CAGGCTCTT | 21201 | TCGGCCGGA AGGCGAGAC ATAAAGAGC AGGACATC ATCAACACT GCGGCATGG GCTCCATGA GCAGGAATG AAGCCATG TCAMAGTCT | 21301 | AGCGCGCCT TCGGTGTG TTATTTCTG TTCTGTAG TTCTGTGA TTCTGTGA CCGGTATC CCGGTATC CCGGTATC CCGGTATC | 21401 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 21501 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 21601 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 21701 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 21801 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 21901 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 22001 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 22101 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 22201 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 22301 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 22401 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT | 22501 | AGGACACCC GGTATGAGT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT GATGCTCTT |
|-------|--|-------|--|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|-------|---|

Figure 15N

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| 22601 | ATCTTGCCCT TGTAGACTG CTCTTCACG GGTGCTATG CTTTCTGCT GTACATCC ATTTCANATCA CGTCTCTCTT ATTTATCATTA ATGCTTCTCTT TAGAACCCGGA ACCATCTGAC GATGATATCG GGTGATATCG GGTGATATCG GGTGATATCG GGTGATATCG GGTGATATCG GGTGATATCG |
| 22701 | GTAGACACTT AAGCTGCTT TCGATTCTG AGCTAGACTG GGTGCTCCAC GTTATCTTG GTGCTATAC GGTGCTATAC TGGCTCTG ATGCTTCTG ATGCTTCTG CATCTGTGTA TTGAGCCGA AGCTAGACTG GGTGCTCCAC GTTATCTTG GTGCTATAC GGTGCTATAC TGGCTCTG ATGCTTCTG ATGCTTCTG |
| 22801 | CAGGTACGCC TGCAGGAATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GTCCATGCGG ACCTGCTTAG GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC |
| 22901 | CATAGCGCGG CAGAGACTTC CACTTCTCA GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GTATGCGCGG GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC |
| 23001 | CCATGCGCTT CTCCACGCA GACGCTATC GACGCTATC GACGCTATC GACGCTATC GACGCTATC GACGCTATC GACGCTATC GACGCTATC GTATGCGCGG GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC |
| 23101 | CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC |
| 23201 | ACCATTTGTA GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC TGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC |
| 23301 | TCCTGCGCGG ATGCTGCTATC ATGCTGCTATC ATGCTGCTATC ATGCTGCTATC ATGCTGCTATC ATGCTGCTATC ATGCTGCTATC ATGCTGCTATC AGAACCCCGG TTACTGCTATC TTACTGCTATC TTACTGCTATC TTACTGCTATC TTACTGCTATC TTACTGCTATC TTACTGCTATC TTACTGCTATC |
| 23401 | CTGCTATACC CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA CCGCTATCCA GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC |
| 23501 | GCACCGCTC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC |
| 23601 | AGAGGACAG CTTAACCCCG CTTAACCCCG CTTAACCCCG CTTAACCCCG CTTAACCCCG CTTAACCCCG CTTAACCCCG CTTAACCCCG TCTTCTCTTC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC |
| 23701 | GAAGGAGGA GTGCTATC GTGCTATC GTGCTATC GTGCTATC GTGCTATC GTGCTATC GTGCTATC GTGCTATC GTGCTATC CTTCTCTCTT CACTATATAC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC |
| 23801 | GAAGGAGGA AGTCTGCGG AGTCTGCGG AGTCTGCGG AGTCTGCGG AGTCTGCGG AGTCTGCGG AGTCTGCGG AGTCTGCGG GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC |
| 23901 | GGGCTATTTT CTGCTATC CTGCTATC CTGCTATC CTGCTATC CTGCTATC CTGCTATC CTGCTATC CTGCTATC CTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC GCTGCTATC |
| 24001 | ACCCGCTTTC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC GGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC TGTGCTATC |
| 24101 | TTTTTCCAAA ACTGCTATC ACTGCTATC ACTGCTATC ACTGCTATC ACTGCTATC ACTGCTATC ACTGCTATC ACTGCTATC ACTGCTATC AAAAAGTTT TGACCTTCTA TGACCTTCTA TGACCTTCTA TGACCTTCTA TGACCTTCTA TGACCTTCTA TGACCTTCTA TGACCTTCTA |

Figure 150

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| | |
|-------|---|
| 25701 | GGGGCTTTGC TTCCAGGAT GGCACCTAAA AAGAACTTTC ATTCTTTTC GTACCCACAG CACGAGGAGG ATACTTGGCA CATCTAGGCA GAGTATGTTT CCCCGGAAGG AAGGGTCTTA CCGATTTT TTCTTCGAG TTAAATGAGG CTTATGATTC CTGCTCTCC GTCTAGTCCCT GTCTCTCCA . |
| 25801 | TGACAGAGGA GAGAGAGGAC ATGATGAGG ACTGAGGAG GTTAAAGAG GAAGCTTCCG GAACACGCT GACCTCTG . ACCTGCTCTT CTTCTCTCTG TACTACTTTC TGACCTCTTC GATCTGTC: CTTGGAAGGC TCACAGTTCT CCACAGTCTG CTTTGTGGCA GTGTAGGCA |
| 25901 | CGCATTTCCC TTGCGCGGCG CCCAGAAATC GGCAGACGAT TCCATCATG CTACACCTTC CTTCTCTCAG CACTGCGCGG CACTGCGGCT TTGCTGAGACC |
| 26001 | GCCTAAGGCG ACGGCGCGG GGTCTTTAG CCGTTTGGCA AATTGCTACC GATTTTGGAG GCGAGGATC GCGCGCGG CTGACGCGCA ACCGCTCGG |
| 26101 | AACCTAGAT GGAACACGAC TGGAACGAG CCGGTAGT CCAAGCAGCC GGTGCTTTTA GCGCAGAGC AACACAGCG CCAAGGCTAC CTCTCATG . TTGGCATCTA CCGTGTGCTG ACCTTGCTC CGGCATTCA GGTTCGTGCG GTCGTCAT TCGGTCCTG CCGGTTCTCG TTGTTGTGCG GGTTCGATG GCGATGACCG |
| 26201 | CGCGGCACAA GAACGCCATA GTTCTTGCT TGCAGACTG TGGAGCAAC ATCTCTTCG CCGGCGCTT CCGGCGCTT TCTTCTCTAC CATTACGCGG TGGATTCTT CGCGCTGTT CTTGGGTTAT CACGAGCGA AGTTCTGAC ACCCTGTTG TATAGGAAAG GCGCGCGGA AGAAGATG GTAGTCCCG ACCTGAGG . |
| 26301 | CGCATTTACT CTGCAATTA TGGCAGTAG GATTCGGGT CTACAGCCCA TACTTACAT GGTCTGCGC GCGCTTCTG TCGCGCGTGT GTCTTCTT CCGTGGCT TACGAGACT CTGACAGC CCAAGAACT CACAGCGCG GAGTACAG GAGTACAGC CTCTCTCTG CCGGCGGGA CAGAACGAA GGTCTGCTT CCGTGGCT |
| 26401 | ATCGTCTGA GACTTTTTC GATTTTTC CACTCTAT CTATATTTC AACAGAGAG GCGCCAGAA CAGGACTGA AATATAAATA CAGGCTCTG CCACTCTCA GATCTTTGT CCAATAAAG GTGAGACATA CGATATAAG TTCTCTCTC CCGGTTCTT GTTCTGACT TTTATTTTT GTCCAGAGC OCTAGCGAT |
| 26501 | CCGCGAGCT CCGTATCAC AAAGCGAG ATCACTTCG GCGTCTGAC CCGTCTGAC CTTCTGCGC TCCGAGAGA GTCATTTAT ACCTGACT GATAATTC . GGGCTCGAC GACATAGTG TTCTGCTTC TAGTGAAGC GCGTCTGAC CCGTCTGAC CTTCTGCGC TCCGAGAGA GTCATTTAT ACCTGACT GATAATTC |
| 26601 | CTAGTTTTC GCGCTTCTC AATTTTAC GCGTCTGAC CCGTCTGAC CCGTCTGAC CTTCTGCGC TCCGAGAGA GTCATTTAT ACCTGACT GATAATTC . GATCAAGCG CCGGAAAGG TTTAATTC GCGTTTGTAT GCTAGTAGG TCGCGCTGT ACCTGCTCA CCGCGGCTT TCCGAGAGA GTCATTTAT ACCTGACT GATAATTC |
| 26701 | GAATTTCCA CCGCTTACAT GTGGATTAC CAGCCACAA TGGTACTTC GGTGAGCT CCGCAGACT GCGCAGACT ACTCAACCG AATAAATAC ATGAGCGCTG CTTTAAGGT CCGGATGTA CACCTCAATG GTCGGTGTT ACCTGAGC CCGACCTGA CCGTCTGTA TGAGTTGGC TTATTGTATG TACTCGGCG |
| 26801 | GACCCACAT GATATCCCG GTCAACGGA TACGCGCCA CCGAACCA ATTCTCTTG AACAGCGC TATTACCAC ACACCTCTTA ATACCTTAA CTCGGCTGA CTATAGGCG CAGTTGCTT ATGCGCGGT GCTTTGCT TAAAGAGC TTGTCGCGG ATATGCTG TGTTGAGCAT TATTGGAAT |
| 26901 | TCCCGTATG TCGCGCGCTG CCGTGTGA CCGAGCAT CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA |
| 27001 | TCAGGCGCG AGCTTGGCG GCGCTTGT CACAGGTC GATGCGCGG GCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA |
| 27101 | AGTCCCGCG TCBAACGCG CCGGAAAGC GTGCGCAGC CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA |
| | ACCTGCTGTT GAGTCTCTG CTGCTCTCC CTTGCTCTC GATATTGAG ATGCGCGG CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA CCGCTCTCA |
| 27201 | TCGCGAGCC TGTCTCTCTG AGCGGCTC TGGAGCAT TGGAGCTC AATTATTA GAGTTTGT CCAATGCT ACTTTAAC CTTCTCGGTA AGAGCTCTG AGGAGAGAC TCGCGCGAG ACCTCGTAA CTTTGAAGT TTAATTAAT CTTTGAAGT CTTTGAAGT TTAATTAAT CTTTGAAGT CTTTGAAGT |

Figure 150

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| | | | | | | | | | | |
|-------|------------|-------------|-------------|-------------|------------|-------------|------------|-------------|------------|-------------|
| 27301 | CTCTCCGCCC | ACTATCCGGA | TCAAATTAAT | CTTAACCTTG | ACGTTATAMA | GGACTGCGCT | GACGCTACG | ACTGAAATGTT | AAGTGGAGAG | GCAGACCAAC |
| | GGAGGCGCGG | TGATAGGCGT | AGTTAATAMA | GGATTGAAAC | TGCTATATTT | CTTCAGCGCG | CTGCTCATGC | TGACTTACAA | TTCACTCTCT | CGTATCTGTT |
| 27401 | TGCGCTTGAA | ACACTGGTTC | CACCTGCTGC | GGCAGACATG | CTTTGCTGCG | GACTTCGCTG | AGTTTCTCTA | CTTTGAAATG | CCCGAGGATC | ATATCTGAGG |
| | ACCGGACTTT | TGTGGACGAG | GTGACATCGG | CGGTGTTTAC | GGAACTGCGG | CTGAGGCGAC | TCAAACGAT | GGAACTTAA | GGCTCTCTAG | TATAGCTCTT |
| 27501 | CCCGGCGCAC | GGCTGCGCG | TTACCGGCGA | GGAGACCTTT | GGCTGACGCT | TGATTGCGGA | GTTTACCGAG | CGCGGCTCTG | TAGTTGAGCG | GGACAGCGTA |
| | GGCGCGCGTG | CCCGAGGCGG | AATGGCGGTT | CCCTCTCGAA | CGCGCATCGG | ACTAAGCGCT | CAAAATGGTC | GGGGGGAGCG | ATCAACTCGC | CGTGTCCCTT |
| | | | | | | Fig.11 | | | | |
| 27601 | CCCCTGTGTC | TCACCTGTAT | TTGCACTGTT | CTTAACCGTG | GAATACATCA | AGATCTTTGT | TGCGATCTCT | GTGCTGAGTA | TAAATATATC | AGAAATTAAT |
| | GGGACACAGG | AGTGACACCTA | AACGTTGACA | GAATGGGAC | CTAATTTAGT | TCTAGAAACA | ACGGATAGA | CACGACTCAT | ATTATTTATG | TCTTTAAT |
| 27701 | ATATACTGCG | GTCTCTATCG | CCATCTGTTA | GAATGACCG | TTTTCACCGG | CCAAAGGCAA | CCAGGCGCAA | CCCTTACCTG | TACTTTTAC | ATCTCTCC |
| | TATATGACCC | CGAGGATAGC | GGTAGGACAT | TTGGGTGCG | AGTAGTGCG | GGGTTGCGTT | GGTTCCGCTT | GGAAATGACC | ATGAATATG | TAGAGAGGTA |
| 27801 | CTGTGATTTA | CAACGTTTC | AACCGAGCG | GACTGAGTCT | ACGATGAGAC | CTCTCCGAGC | TCACCTACTC | CATCAGAAAA | AACACACCC | TCTTACCTT |
| | GGGACACAGT | GTGTGCAAG | TTGGGTCTGC | TTGGGTCTGC | CTACCTCAGA | TGCTCTCTTG | GAGAGGCTCG | AGTGGATGAG | GTAGTCTTTT | TTGTGGTGG |
| 27901 | CCCGGACGCT | AGGAGTGGCT | CACCGGCGC | TTGACCCAC | CTACCGCTG | ACCGTAAACC | AGACTTTTTC | CGGACAGACC | TCATTAACCT | TGTTTACCGT |
| | GGCGCTTGCA | TGCTCACGCA | GTGCGCGCGG | ACGTGGTGTG | GATGGGAGAC | TGGCATTTGG | TCGTAAGAG | GGCTGCTCTG | AGTTATTTAG | ACAAATGGTC |
| 28001 | AACAGAGGCT | GGCTTTAGAA | AACGCTTAGG | GTATTTAGCG | AAATGCGCAG | CTACTGTGTG | GTTTATGAGC | ACTCTACCGG | ACTCTACCGG | CTATCTCTAA |
| | TTGTCTCTCA | CTCGAATCTT | TTGGGATATC | CATPATCCCG | TTTCCGCGTC | GATCAGACCC | CAATATCTTG | TTAAGTTGCT | TGAGATGCGC | GATATGATTT |
| | | | | | | XbaI | | | | |
| 28101 | TCAGGTTTCT | CTAGAAATCG | GGTTGGGGTT | ATTCTCTGTC | TTGTGATCTT | CTTTATCTCT | ATACTAACGC | TTCTCTGCGT | ANGGCTGCGC | GGCTGCTT |
| | AGTCCAAAGA | GAATCTTACC | CCAAACCCCA | TAAGAGACAG | AACACTAGAA | GAATATAGAA | TATGATTTGG | AAGAGACGGA | TTCCGAGCGG | CCGAGGACAC |
| 28201 | TGCACATTTG | CATTTATTTG | CAGCTTTTAA | AACGCTGCGG | TGCGCACCCA | AGATGATTTAG | GTATCTAATC | CTAGGTTTAC | TCACCTTTGC | GTACGCTCCAC |
| | ACGTGTAAAC | GTAAATACAA | GTGGAATAAT | TTGCGACCCC | ACCGTGCGGT | TCTACTAATC | CATGATTTAG | GATCCAAATG | AGTGGGAAAG | CAGTGGGTT |
| | | | | | | KpnI | | | | |
| 28301 | GGTACCCACC | AAAGGTGGA | TTTTANGGAG | CCAGCTGTTA | ATGTTACATT | CGACGCTGAA | GGTAAATAGT | GCACCACTCT | TATATAATGC | ACCACAGN |
| | CCATGGTGCG | TTTTCCACCT | AAATTCCTTC | GGTGGGACAT | TACATCTTAA | GGCTCGACTT | CGATTACTCA | CGTGGTGAGA | ATATTTTACG | TGGTGTCTTT |
| 28401 | ATGAAAGCT | GGTATTTGCG | CACAAAGCA | AAATGGGCAA | GTATCTGCTT | TATCTATTT | CCAGGCGCAG | TCACACTACA | QAGTATATG | TTACAGTTT |
| | TACTTTTTCG | CGAATAGCG | GTGTTTTTGT | TTTAAACGCT | CATACGACAA | ATACGATAMA | CCGTGCGTCC | ACTGTGATGT | CTCATATTAC | AATGTCABAA |
| | | | | | | Bst107I | | | | |
| 28501 | CCAGGGTAAA | AGTCATPAAA | CTTTTATGTA | TACTTTTCCA | TTTTATGAAA | TGTGCGACAT | TACCATGTAC | ATGAGCAAAC | AGTATAGTT | GTGCGCCCA |
| | GGTCCCATTT | TCAGTATTTT | GAATAATCAT | GAATAATCAT | AAATATCTTT | ACACGCTGTA | ATGGTACATG | TACTGTTTTG | TCATATTCAA | CACCGGRT |
| 28601 | CAAAATTTG | TGAAACAC | TGGCACTTTC | TGCTGCACTG | CTATGCTAAT | TACAGTCTC | GTCTTGCTCT | GTACCTTACT | CTATATTAAA | TACANAGCA |
| | GTTTTATAC | ACCTTTTGG | ACCGTGAAG | ACGAGGTGAC | GAATGATTTA | ATGTCAGGAG | CGAAACCGAA | CATGGATGA | GATATATTTT | ATGTTTCTT |
| 28701 | GACGACCTTT | TATTGAGGAA | AGCAAAATGC | CTTAAATTTAC | TANGTTACAA | ACTAATGTC | ACCACTACT | GGTTTACTCG | CTGCTTGCAA | AACAAATTT |
| | CTGCGTCAA | ATACTCTCT | TTCTTTTACG | GAATTAATG | ATTCATCTTT | TGCTATTACG | TGCTGATTA | CGAAATGAGC | GAACAACTTT | TTGTTTAAAT |
| 28801 | AAAGGTTAGC | ATTATATTTA | GAATAGGATTT | TAAACCCCTC | GGTCAATTTG | TGCTCAATAC | CATTCGCCCT | ACAAATTTGAC | TCATGCTGGG | ATATCTCTA |
| | TTTTCAATCG | TAAATATTAA | CTTATCTTAA | ATTTGGGGGG | CCAGTAAAGG | ACGAGTTATG | GTAAAGGAGC | TTGTTTACTG | AGATACACCC | TATACCGAGT |

Figure 1SR

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28901 GCGCTACAC CTTGACATCA GCTTCTCTG AGCTAGCAT CTACTTTTG CAGGACCTG TCCGCGCAT TTGTTCCAGT CCACTACAG CCACCCACATC
 CCGCATCTG GAACTTCAGT CCGACAGATC TACAGTCTA GACTGAAAC GGTGCTGAC AGGCGCTTA ANCAAGTCA GGTTCATGTC GCTTATATC
 29001 TAACAGAGAT GACCAACACA ACCAACGCG CCGCGCTAC CCGACTTACA CTACACACA ATACACCCA AGTTCTGACC TTGTCATAA ACTCGGATAA
 ATTCTCTCA CTGCTCTGT TGTTCGCGC GCGCGCATC GCTGAAATC GCTGAAATC AGATGCTCT TATGTTGCGT TCANAGACCG AACAGTTAT TCACCTCAT
 29101 CTTCGECAG TGTGCTCTT CCATAGCTT TATTTTCTA TGTCTTATA TGTGTTAT TATGTTAT CATCTCTGC CTAAAGCGCA AACCGCGCG ACCACCCATC
 GAACCCCTAC ACCACCAAGA GCTATGCGA ATACAAACAT ACCAATAT ATACACCTA GAGACGCTG AAACACATG TCTTTCTCT TACAGTATGA TTAATAGTGA
 29201 TATATGCCA TCATTTGCT ACACCCAAAC AAATATGTA TGTATAGAT GAGACGCTG AAACACATG TCTTTCTCT TACAGTATGA TTAATAGTGA
 ATATCAGGCT AGTAAACAGA TGTGCTCTT TACTACTCTT AGATATCTA CCGCTCTGAC TTGCTGACA AGAAAGAGA ATGTCATCT AATTACTCT
 29301 CATGATCTT CAGTTTTTA TATTACTGAC CTTCTCTG CTTCTCTG CTTCTCTG ATGCTCTG GTTCTCTG TCGAAGTGA CTGATCTC A
 GTACTAAGA GCTCAAAAT ATATGACTG CCAACACCG CAACAAACAG CAACAAACAG TACCGACCG CAAGAGTGT AGCTTCATCT GACGTAGT
 29401 GCTTCTACAG TCTATTTGCT TTACGATTT GTACCCCTCA CCGCTCATCT CAGCTCTG ACTCTCTCA TCGCTTTAT CCACTGCTT GACTGGCTT
 CGAAGTCTC AGATAACGA AATGCTTAA CAGTGGGAT GCGAGTACAG GTCTGATGAG TCGACCATG AGCGAATAA GGTACAGTAA CTGACCCCA
 29501 GTGTGCTCT TGCATATCT AGACACCAT CCGCATACAG GAGACGACT ATAGCTGAG TTCTTGAAT TCTTTATTA TGAATTTAC TGTGACTT
 CACAGCGAA ACTATAGAG TCTGCTGAG GGTCTATG CCTGCTCTG TATGACTG AGAATCTTA AGAATTAAT ACTTTAATG ACACCTAATA
 29601 CTGCTGATTA TTGCACTCT ATCTGCTTT TGTTCCTCG CTTCCACCG TCMAAGCAT ATATCACTG GATTCACCTG TATATGAT TATTCAGIT
 GACACTAAT AACCTGCGA TAGACCGAA ACAGCGGCT GGAAGTCTG AGTTCTCTA TATAGTCTT CTAGTACG ATATACCTTA TAAGTTCTA
 29701 GCPACATGA AAAAAAGT CTTTCCAG CTTGCTTATA TGTATCTG TGTCTCTG TACCATCTA GCTTACCTA TATATCC A
 CATGTTACT TTTTCCCTA GAAAGCTTC GAACTAAT ACTTTAGTAG AGACAATAG ACAGAGCTG ATGTAGAT ATGTAGAT ATATAGAT
 29801 CTTGNCAT GCTGAGAG CAATGATG CATGACAC CCACTTTTC CCGCGCTG TATGCTTCA CTGCAACAG TTGTTGCGG CCGCTTCTG
 GAACTGTA CCACTTCT GTTATCTAG GTACTTGTG GTTGAAGA GCGCGCGCG ATACGAGGT GAGTTTCT ACACAGCGC GCGAATAA
 29901 CCAACCATC AGCTCTGCT ACCTCTGCT TGGAGAGAGT AATACAGCTA CTTTAATCTA ACAGAGAG AGACTGACA CCGTACATCT AGAATGAC
 GGTGCTTAT TCGAGAGAG TGGGCTGAC TTTATCTAT GAAATTAAT TGTCTCTCT TACTACTCT TACTACTCT GCGATCTAGA TCTTTACTG
 30001 GGAATTAATA CAGAGCAGG CTTGCTAGAA AGACGAGAGT CAGCGCTGA GCAACAGCG ATGAATCAG AGCTCCAGA CATGTTTAC TTGACCCAGT
 CTTTAATAT GTCTGCTG GAGAGATCT TCTGCTGCT CTTGCTGCT TACTTATCT TCGAGTTCT GTACCAATG AACCTGCTA
 30101 GCAAGGCGT TATCTTTCT CTGTAAGC AGCTACGAT CACTACGAT AGTATACCA CCGGACCG CTTTACTAC AGCTGCGAA CCAAGCTTA
 GGTCTCTCC ATAGAAACA GAGCATTTG TCGGCTTCA GTGATGCTG TCAATATCT GCGCTCTG GGAATGATG TTCAACGCTT GGTCTCTAT
 30201 GAATGCTG GTCATGCTG CAGAAAGCT CATACCAT ACTACGACT CTATAGAAC CTATAGAAC CCAAGCTGCTT TCGTCTGAGT ACCTGAGT
 CTTTAMCCAC CAGTACCAC CTCCTTCTG GTATATGAT TATGCTGTA GCTATCTG GCTTCTGAGT TATGAGT GACAGTTCT TCGACTCTA
 30301 CTCTGACCC TTATTAGAC CTTGCTGCT TATCTCTCT TACTATTA TAAATTA TAAAGTCA CTACTTAA ATCAGTTAG
 GAGAGCTGAG AATATTTCT GAGACACCCA GAGTTCTAG AATAGGTA ATTATAT TTTTTTAT ATTCTGAT GAATGAAT TATGTAATG

Figure 155

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30401 AAATTTCTGT CCAGTTTATTT CAGCAGCACC TGCTGTGCTT CCTTCCAGCT CTGTATTGTC AGCTTCTCC TGGTGCAAA CTTCCTCCAC AATCTAAATG
 TTTAAAGACA GGTCAATATA GTGCTGCTGG AGAACGGGA GAGAGGTCCA GACTATATAG TTGMAAGGG ACCGACGTTT GAAAGAGGTG TTAGATTATC
 GAATGTCAGT TTCTCTCTGT TCTGTCTCAT CCGTACCCAC TATCTTCATG TTTTGTATGA TGAAGGTGTC AATGACCTCT TCAACCCCTT
 CTACAGTCA AAGCAGACA AGCAGAGTA GGTGTGTG ATAGAGTAG AACAGCTCT ACTGTGCGG TTCTGTACGA CTCTATATGA AGTTAGGTG A
 30601 GTATCCATAT GACACGAAA CCGTCTCC GGCAGGAG GTACACGGA AAGAGTAG AGGGGTTA TGGGAGTTT CCAAGAGTTT TCTCAGGGGG ACCCCATGAG
 CATAGGTATA CTGTGCTTTT GGCAGGAGT
 30701 TCTTTGCCCC TATCCGAACT TCTATTTACC TCCATATGGA TCTTTGCTT CAAATGTC AACTGCTCT CTCTGAGGA GGCCTGTCAC CTTCACCTCT
 AGAAGCCGG ATAGGCTTGG AGATCATATG AGCTTACCT AGTACGCTGA GTTTTACCG TTGCTGAGGA GAGACCTGCT CCGGCGGTTG GAATGAGT
 30801 AAATGTATAC CACTGTGAGT CCACCTCTCA AAAAACTCA GTTAAATATA AACTGTGAAA TATCTGTACC CCTCAGAGTT ACCTCAGAG CCECTACTCT
 TTTTACATGG GTACACTGG GTTGTAGAT TTTTGTGCTT CAGTTGTAT TTGACCTTT ATAGACCTGG GAGGTGTCMA TGGAGTCTTC GGAATTGACA
 30901 GGTGCCCCC GCACCTCTAA TGTGTGCGG CAACACATC ACCATGAT TGTGTGTTA GTGTGCGGG CGATTGCTAC GTCTGAGGT TTGAATGTA ACCTGCTT
 CCGACCGGG CGTGTAGATT ACCAGCGCC GTTGTGTG TGTGTGTTA GTGTGCGGG CGATTGCTAC GTCTGAGGT TTGAATGTA ACCTGCTT
 31001 GACCCCTCA CAGTGTGAGA AGGAACCTA GCGCTGAAA CATCAGGCC GTATGCTGG GAGTGTGCT TGGCTATGCT CATGTAGCTG ATGTGCGGA
 CCTGTGAGT GTACAGGCTT CCGGAGCTTT GTATGCTGG GAGTGTGCT TGGCTATGCT CATGTAGCTG ATGTGCGGA
 31101 TAACTACTGC CACTGTGAGT TTGGCATGG ACTGTGAA GACCATTTAT ACACATATG GAAACTAG ACTAAGTAT GGGGCTCTTT TGTATGTA
 ATGTATGAGC GTGTACATGG AACCGTAC TGAATTTCT CCGGTAAATA TGTATTTTAC TGTATTTTAC TGTATTTTAC TGTATTTTAC TGTATTTTAC
 31201 AGACAGCTTA AACTATTTGA CCGTAGCAAC TGGTCAGT GTCATATTA TGTATTTTAC TGTATTTTAC TGTATTTTAC TGTATTTTAC TGTATTTTAC
 TCTGTGATG TTGTGAACT GGCATCTTG ACCAGTCCA CACTGATAT TATTATGAG GAAGTTTGA TTTCAATGAC TTTCAATGAC TTTCAATGAC
 31301 CAAGCCATTA TGCACCTTAA TGTAGCAGA GGCATTAAGA TGTATTTTCA TGTATTTTAC TGTATTTTAC TGTATTTTAC TGTATTTTAC TGTATTTTAC
 GTTCCGTTAT ACCTGAAAT ACATGCTCT CCGTATTTCT ACTAAGAT TTTGTGCTG GAAATGAACT TACATCAAT AGGCAAACTA CAGCTTCAAA
 31401 AACTAAATCT AAGACTAGGA CAGGCTCTC TTTTATANA CTCAGCCCAE AACTGTGATA TTAACATCA CAAAGCCCT TACTTGTTTA CAGCTTCAAA
 TTGATTTAGA TTTGATTTCT GTCCCGGGAG AAAAAATTT GAGTGGGGTG TGTAACTAT AATGATGTT GTTTCCGGA ATGAACTAAT GTCCAAATTT
 31501 CAATTCGAAA AAGCTTGAGG TTAACTTAAG CACTGCCAG GGTGTGATGT TTGACCTTAC AGCCATGCT ATTAAATGAG GAGATGGCT TGAATTTGTT
 GTTAGGTTT TTGAACTCC AATTTGATTC GTGACCTTC CCGACTTCA CCGACTTCA CCGACTTCA CCGACTTCA CCGACTTCA CCGACTTCA
 31601 TCACTTAATG CACCAACAC AATCCCTC AAAAAA TTTTCCATGG CCTAGATTT GATTCAGCA AGCTATGCT TCGTATGCT TCGTATGCT TCGTATGCT
 AGTGATTTAC GTGTTTGTG TTTAGGGGAG TTTTGTGTTT ACGGCTACC GGAATCTTAA CTAACTTAA CTAACTTAA CTAACTTAA CTAACTTAA
 31701 TTAGTTTGA CAGCAGAT GTGATTTAG GGTATGCT AATTAATGAT AACTAATCT TTTTATTTA TTTTATTTA TTTTATTTA TTTTATTTA
 AATCAAACT GTGTTGCTA CCGTATGCT AACTTGTGTT TTTTATTTA TTTTATTTA TTTTATTTA TTTTATTTA TTTTATTTA TTTTATTTA
 31801 TGCAGGAAA GATCTTAAC TCAGTTTGT GTTAAACAAA TGTGCTGCT AATATTTTGT TACATTTTCA CAGCTTCTCA GTTTTGTGCT TTTTGTGCT
 AGTCTCTTT CTACGATTT AGTGAACCA GAAATGTTT GAAATGTTT ACAGCTGAG TTTATGAGG ATGTCAAGT CAAAGCCGAG AATTTCCGCT AATTTCCGCT
 31901 ATATCTGAAA CAGTTTCAAG TGTCTATCT ATTAAGAT TTTTGAAGA TTTTGAAGA TTTTGAAGA TTTTGAAGA TTTTGAAGA TTTTGAAGA
 TATAGACCTT GTCAAGTTTC ACGATGAG AATATTTCTA AACTGCTTTT AACTGCTTTT AACTGCTTTT AACTGCTTTT AACTGCTTTT AACTGCTTTT
 32001 GAATGTGAGA TCTTACTTAA GGCAGCTCT ATACAAAGC TTTGTATTT ATCTTACC TATGACTTA TCCAAATCT CAGCTTAAAG CTGCTTAAAG
 CTTTACTCT AGAATGACTT CCGTGTGGA TATGTTTGG AACTTAA TACGATTTG ATAGTCAAT AGGTTTNGA GTGCCATTTT GAGGTTTCT

Figure 15T

pMRKA15:qag MER6B2

32101 TAACATATGC AGTCAGATT ACTTAACGG ACACAAACT AACCTCTTA CACTAACAT TAACTTAAC GTACACAGG AACAGGAG CACAACTCA
 32201 ATGTAAACAG TCAGTTCAAA TGAATTTTGC TCTCTTTTAA TTTTAACAT GTATTGTGA ATGTAAATTC CCATGTCTCT TTGTCTCTCT GTGTTAGAT
 32301 AGTGCATACT CTATGTCAAT TTAATGTGAC TATCTGTGAT ACATTAAT TAATTAATA TTTTTCATAC CTCTTTACAC ATTTTCATAC ATTGCCANV
 32401 TCACATATGA GATACAGTAA AGTACCTCTG ACCAGACCGG TCTTAATTA ATTACTTTAT AACCTGTGA GAGAAATGTC AAAGATATG TAACGCTTT
 32501 AATAAGAAAT CGTTGTGTT ATGTTTCAC GCTTTTATTT TCAATTTTA GAAATTTCA AGTCAATTTT CATTCAGTAG TATAGCCCA CCACACATA
 32601 TTAATTTCTTA GCAACACAA TACAAGTTG CACAATATA AAGTAAAGT CTTTAAGT TCAATTAATA GTAACTCAT ATATCGGCT TTGTTCTTA
 32701 GCTTATACAG ATCACCGTAC CTTAATCMA CTCACAGAC CTTAGTATTC AACCTTCAC CTCTCTCCA ACACACAGAG TACACAGTCC TTGTTCTTA
 32801 CGAATATGTC TAGTGCAAT GAAATTAATTT GAGTGTCTTG GATACATAG TTGAGCGTG TTGAGCGTG CACGCTTTC CAGCTTTC AGTATAT
 32901 GCTGGCTTAA AAAGCATCA TATCATGGT AACAGACATA TTCTTAATTC TTAATTTCA CAGCTTTC ACAGCTTTC TTGAGTAG TCACTATM
 33001 CGACCGAAT TTTTCGTAGT ATAGTACCA TTCTCTCTAT AAATATCCAC AATATAAGT GTGCCAAGG ACAGCTTTC TTGAGTAG TCACTATM
 33101 ATAAACTCC CGGCGAGCTC ACTTAAGTTC ATGTGCTGTT AGCCACAGG ACCACAGG TCTGTCTCAA CTGCTCTCAA GTTACCGGC GCTTAAGTA
 33201 TATTGAGGG GCGCTGCGAG TGAATTCAG TACAGCGACA GTTGAGTAC TGGGTGCTG AGCAGAGTT GAACCGCAG GAACTGCCG CCGCTTCT
 33301 AAGTCCACCG CTACATGGG GTAGATCAT ATGTGCTAT CAGGATAGG CCGTGTCTCT GCAGAGGTC GCGATTAAC TCTGTGCCG GCGCTTCTCT
 33401 TTCAAGTGG GATATACCCC CATCTAGTA TTACACCTA GTCTATGCC GCACACCGA CCGTGTGCG CCGTATTTG ACAGCGCGG CCGCGAGCA
 33501 CCTGCAGAA TACAACAGG CAGTGTCTC CTCAGCGATG ATTCGACCG CCGCGAGCT AAGCTGCTT GTCTCTCGG CACAGCAGG CACGCTGAT
 33601 GGACGTCTCT ATGTTGTACC GTACACAGAG GAGTGTCTAC TAAGCTGTC GCGCTGCTA TTCTGCGCA CAGAGCGCC GTGTGCTGC GTGGAGT
 33701 TCACTTAAT CAGCAGTA ACTGCAGAC AGCACCAGAA TATTTTCAA ATCTCCACG TGCAGGCGC TGTATCCAA GTCTATGGG GCGACACAG
 33801 AGTGAATTTA GTGTGTCTAT TGACGTCTG TGTGTGTTT ATACAGATTT TTAGGCTCTC AGTGTCTCG ACATAGTTT CCGTACCGC CCGTGTGTC
 33901 AACCCAGCTG GCAATCATAC CACAGCGCA GTTAGATTAA GTGCTGCTC CTCATACCA CCGTGTGAT AACATTAAC TCTTTTGGCA TGTCTTAT
 34001 TTGGTGCAC CCGTGTATG GTGTTGCGT CCATCTAAT CACGCTGCG GAGTATTTGT GCGACCTGTA TTGTATATG AGAAGCGCT ACACATTA
 34101 CACACCTCC CGTACATA TAACTCTG ATTAAACAT GCGCATCA CACATCTT AACCCAGCT GCGAAGCT GCGCGCGCG TATACACT
 34201 GTGGTGGAG GCAATGCTAT ATTTGAGAC TAATTGTAC CCGGTAGGT GTGTAGGA TTCTGTGAC CCGTGTGCG CCGCGCGCG ATATGTGAC
 34301 AGCGAACCG GACTGGAACA ATGACAGTG AGAGCGCGG ACTGTACCC ATGTATATC ATGTATATC TCAATATCA TCAATATCA CACAGCGCA
 34401 TCGCTTGGC CTGACCTTT TACTGTGACC TCTGCGGTC TGACATATG TACCTAGTAG TACGAGCAT ACTATAGTA CACCGCTT GTGTGCTGT
 34501 CCGCTATACA CTGCTTACG ATTAACAT CTCTGCGT TCCAGGGA CAGCCATTC CTGATACG GTAAATCCA CAGTACAGG CAGTACAGG
 34601 GCACTATCT GAGAGTCC TAATGTTCA GAGAGCGCA ATCTGTAT AGGTGCTT GTGTGTAG GACTTATG CATTTAGGT GTAGCTGC
 34701 AAGACCTCC ACTTACTCA CTTGTGCTAT TGTCAAGTGT TACATCTCC AGTATCTG AGTATCTG CCGCTGCTT TGTCTCAA
 34801 TTCTGAGCG TGCATTTGAT GCAACGCTA ACATTTTAC AATTAAGCC CTTCTGCTC TACTATAGG TCACTATC CCGCGCAAG ACAGCTTT
 34901 GAGGAGTAC GATCTTACT GTACGATG GCGGATACA ACTGATAG CCGGATAG TTTCTGCTC ATGTATATC CAAATGAG CCGCGCTA GTATATTT
 35001 CCGCTATCT CTAGGATGA CATCTCTAC GCGCTCTGT TGGCTCTAG ACACCGCA TCACTAGG GTTTACTG CCGCTGCT CAGTATTAAT

Figure 15U

pHRad5gag MER6R2

35301 CATTATTAGA AACTACAAAT TCCCAACACA TACAGATTAC TCCCTCTTAA AACCTACGTC ACCCGCCCTGG TTCCGACGCG CCGCGCCACG TCACAAACTC
GTAAATCTT TTTCATGTTA AGGTTGTGT ATGTTCAATG AGCTGKATTT TTGGATCCAG TGGGCGGCGC AGGCGTCCGG GCGCGCGTGC AGTGTTTGAG

35401 CACCCCTCTA TTATCATATT GCCTTCAATC CAAATAAGG TATATTATTG ATGATGTTAA TTATGATTTT GCATCTGCGA CCGCAGCGTG GATCGCCCTT
GTGGGGAGT AATAGTATAA CCGAGATTAG GTTTTATTTCC ATATAATATC TACTACATTT ANTTCTTAAG CCTAGACGCT GCGCTCCGAC CTACCGGAAAG

35501 CCCATTATGA TTCTTTCTGC TTCCCGCGGC ATCGGATTC ATCGGTTTGA CCGGATGCTG TCCAGAGAGG TACATACGGA CCAATCAGGA CAGCTTCAGG
GGTATATACT AAGAGAGCGG AAGCGCGCGG TACGCTTAGG GGGCAAGCT CCGGTACGAC AGGTCGCTCC ATCTACTGCT GTTAGTCCCT GTCCAAAGTTC

35601 GCGAGGAAAA GCGCAGGAAAC CCGCTTTGCT GCGCTTTTTC CATAGCTTCC GCGGAGGAG GTATCCGAGG CCGCGGACT GCTCGTAGTG TTTTATAGTG CCGATTTCAGT
CGTCCCTTTT CCGTTCCTTG GCATTTTCTC GCGGCAAGG GATACGAGG ATATCCGAGG TTTCGCGCTC GATCGCTCT CCTGTTCCGA CCGTCCGCTT TACCGBATAC

35701 GAGGTGCGGA AACCCGACAG GACTATAAAG ATACCAGGCG TTTCGCGCTC GATCGCTCT CCTGTTCCGA CCGTCCGCTT TACCGBATAC
CTCCACCGCT TTGGCTGTC CTGATATTTT TATGTCCTCC AAGTGGGAC CTTCGAGGGA GCGACGCGGA GCGTCCGCTT CCTGTTCCGA CCGTCCGCTT TACCGBATAC

35801 CTGTCCGCTT TCTGCCCTT GCGGAGGCTG GCGCTTCTC ATAGCTCAGG TATCGAGTCC GATTCATCC GATTCATCC TCGTCCGCTT TACCGBATAC
GACAGCGGGA AAGAGGAGAG CCGTTCGAC CCGGAGGAG TATCGAGTCC GATTCATCC GATTCATCC TCGTCCGCTT TACCGBATAC

35901 TCGACGAGCC CCGCTTCTAG CCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC
ACGTGCTTGG GCGGCAAGTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC

36001 CACTGTATAC AGGATTAGGA GAGCGAGGTA TCGGAGGTA CCGGAGGTA GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC
GTGACCATTT TCTTAATCTT CTGCTTCTC ACATCCGCA GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC GCGCTTCTC

36101 ATCTGCTC TCGTGAAGCC AGTTACCTTC GGAAGAGAG TTGTAAGTC TCGTGAAGTC TCGTGAAGTC TCGTGAAGTC TCGTGAAGTC TCGTGAAGTC
TAGACGCGAG ACAGCTTCTG TCAATCGAG CCGTTCCTC GGAAGAGAG TTGTAAGTC TCGTGAAGTC TCGTGAAGTC TCGTGAAGTC TCGTGAAGTC

36201 AGCAGCAGAT TACGCGCAGA AANAAGGAT CTCAGAGAGA TCGTTCGATC AANAAGGAT TCGTTCGATC AANAAGGAT TCGTTCGATC AANAAGGAT
TCTGCTCTA ATGCGGCTT TTTTCTCTA GAGTCTCTT AANAAGGAT TCGTTCGATC AANAAGGAT TCGTTCGATC AANAAGGAT TCGTTCGATC

36301 TTGCTGATG AGATTATCAA AAGGATCTT CAGCTAGATC CTTTAAATC AATCTAAGT ATATATAGT AACTTCTC TACAGTTAC CAACTCTTAA
AAGCAGTAC TCTAATAGTT TTCTCTAGAA GTGGATCTAG GAAATTTAG TTAGATTTCA TATATCTCA TTTGAGCAG ACTGTCAATG GTTACGAAAT

36401 TCGTGAAGGC ACCTATCTCA GCGTCTCTC TATTCTCTC ATCTATAGTT GCGTCTCTC TATATCTCA TATATCTCA TATATCTCA TATATCTCA
AGTCACTCCG TGGATAGAGT CCGTGAAGAG ATAAAGCAG TAGGTATCAA CCGCTCTC GCGCTCTC GCGCTCTC GCGCTCTC GCGCTCTC

36501 TCGCGCCAGT GCTGCATGGA TACCGCGAGA CCGCCTCTA CCGCTCTC CCGCTCTC CCGCTCTC CCGCTCTC CCGCTCTC CCGCTCTC
ACCGGCTCA CCGCTTACT ATCGGCTCT GCGTCCGAGT GCGCGAGTCT TAAATAGTCT TAAATAGTCT TAAATAGTCT TAAATAGTCT TAAATAGTCT

36601 CCTGCACTT TATCCGCTC CATCCAGTCT ATTAATTTGT GCGCGAGTC TATATTAAT ATGTCGCGAG TATATTAAT ATGTCGCGAG TATATTAAT
GAGCTGGA ATAGCGGAG GTAGGTCTAGA TAAATTAACA CCGCGCTCTC ATCTATCTA TCAAGCGTCT TCAAGCGTCT TCAAGCGTCT TCAAGCGTCT

36701 CTACAGCAT CCGTCTCTA CCGTCTCTA CCGTCTCTA CCGTCTCTA CCGTCTCTA CCGTCTCTA CCGTCTCTA CCGTCTCTA CCGTCTCTA
GATGCTCTA GCGCAGAGT GCGCAGAGT AAGTAAAGT AAGTAAAGT AAGTAAAGT AAGTAAAGT AAGTAAAGT AAGTAAAGT AAGTAAAGT

36801 AANAAGGAT AGCTCTCTC GTCTCTCTA GTCTCTCTA GTCTCTCTA GTCTCTCTA GTCTCTCTA GTCTCTCTA GTCTCTCTA GTCTCTCTA GTCTCTCTA
TTTTCGCA TCGAGGAGC CAGGAGCTA GCGCATCTT TCAATCAAC CCGCTCTC TCAATCAAC CCGCTCTC TCAATCAAC CCGCTCTC TCAATCAAC

36901 GTCATGCCAT CCGTAAAGT CTTTCTCTG ACTGCTAGT ACTTAACCA GTCAATCTCA GATATCTCA GATATCTCA GATATCTCA GATATCTCA
CAGTACGTA GCGATCTCTAC GAAAGAGAC TCAACCACTA TCAATCTCA TCAATCTCA TCAATCTCA TCAATCTCA TCAATCTCA TCAATCTCA

figure 15W

pMRKAL59ag MER682

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37001 CAACACGGA TAAACCGG CCACATAGA GAACCTTAA ACCTCTATC ATTGTAAAC GTCTCTGGG GCGAAACTC TCAGGATCT TACTTCTGT
GTGTGCGCT ATTATCGGC GGTGTATGT CTTCGAATTT TACGACTAG TACCTTTTG CAGACGCC CCCTTTTGAG AGTCTCTAGA ATGCGACAA
37101 GAGATCCAGT TCGATTTAC GCACTCTGC ACTGACTTA TCTTAATAT TTTTACTTT CACCAAGCTT TCTGCTGAG CAAACAGCG AAGCGAAT
CTCTAGTCA AGCTACATG GGTGACAGG GTGCTGACT AGAATCTTA GAAATGAAA GTCTGCGCA ACACCCACTC GTTTTCTCC TTCCCTTTA
37201 GCGCCAAAA AGGATATG GCGACACCG AATGCTTGA TACTTACT TTCTTTTT CATTATATT GAGCATTTA TCAGGTTAT TCTCTCATTA
CGCGTTTTT TCCCTATC CCGCTGCG TTACAACTT ATGAGTATA GAGTAAAA GTTATATTA CTTCGTAAAT AGTCCCAATA ACAGAGTAT
37301 GCGATACAT ATTGAAAT ATTAGAAA ATAAACAA ATAAACAA ATAAACAA AGGCTTTC GCTACATTC GCGACCTGAC GTCTAAGAAA CCATTATTA
CGCTATGTA TAACTTACA TAAATCTTT TATTGTTA TCCCAAGC GCGGTAAAG GGGCTTTCA CCGTGGACTG CAGATCTCT GGTAAATATA

                               BamHI
                               vvvvvvvv
                               -----
                               EcoRI
                               vvvvvvvv
                               -----
37401 CATGACATTA ACCTATAAA ATAGCGTAT CACGAGGCC TTGCTCTC AGAATTTGA TCTGATCTT TAAT (SEQ ID NO: 27)
GTACTGTAAAT TGATATTTT TATCCGATA GTGCTCGCG ATAGCAGAG TCTTAACT AGGCTTAAA ATTA (SEQ ID NO: 28)

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Figure 15X

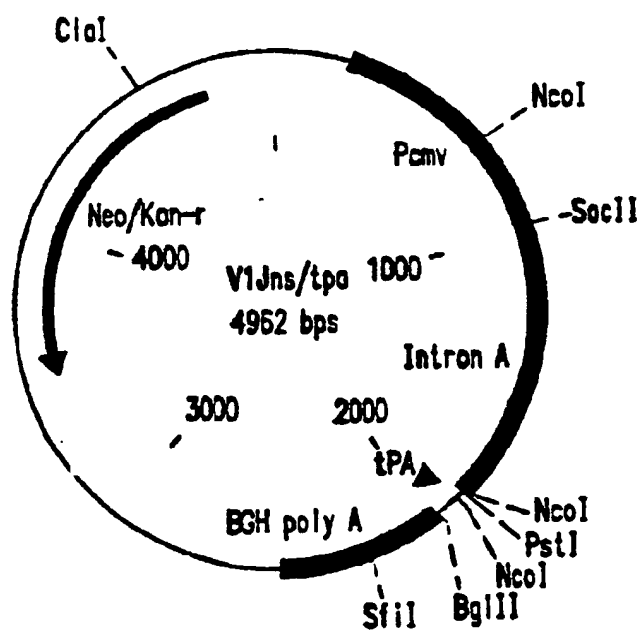
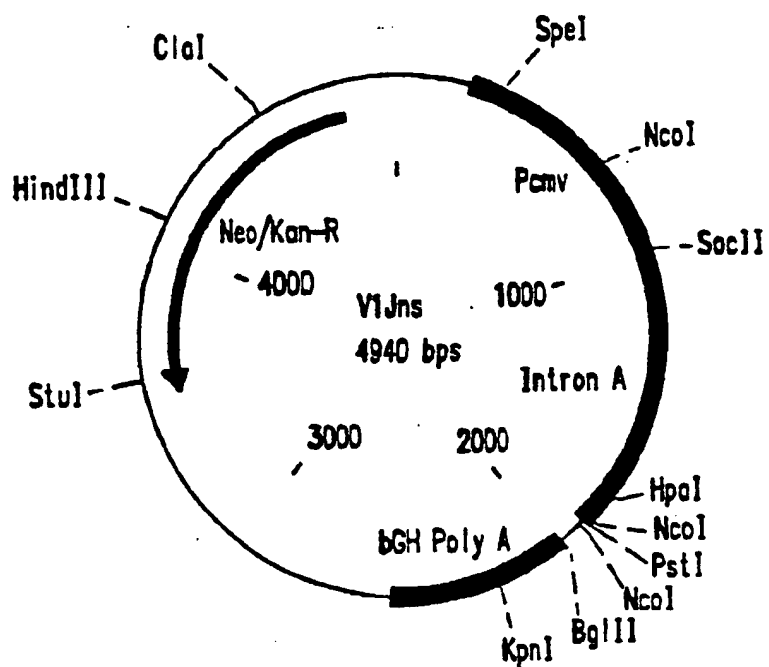


FIGURE 16

AGATCTACCATGGCCCCCATCTCCCCATTGAGACTGTGCTGTGAAGCTGAAGCCTGGCATGGATGGCCCCAAGGTGAA
 Bg/III MetAlaProIleSerProIleGluThrValProValLysLeuLysProGlyMetAspGlyProLysValLy
 1 10 20

GCAGTGGCCCCCTGACTGAGGAGAAGATCAAGGCCCTGGTGGAAATCTGCACTGAGATGGAGAAGGAGGGCAAAATCTCCA
 sGlnTrpProLeuThrGluGluLysIleLysAlaLeuValGluIleCysThrGluMetGluLysGluGlyLysIleSerL
 30 40 50

AGATTGGCCCCGAGAACCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGGACTCCACCAAGTGGAGGAAGCTGGTG
 ysIleGlyProGluAsnProTyrAsnThrProValPheAlaIleLysLysLysAspSerThrLysTrpArgLysLeuVal
 60 70

GACTTCAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCACCCCGCTGGCCTGAAGAA
 AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluValGlnLeuGlyIleProHisProAlaGlyLeuLysLy
 80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGATGCCTACTTCTGTGCCCCCTGGATGAGGACTTCAGGAAGTACACTG
 sLysLysSerValThrValLeuAlaValGlyAspAlaTyrPheSerValProLeuAspGluAspPheArgLysTyrThrA
 110 120 130

CCTTCACCATCCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCACTACAATGTGCTGCCCCAGGGCTGGAAGGGC
 loPheTrnIleProSerIleAsnAsnGluThrProGlyIleArgTyrGlnTyrAsnValLeuProGlnGlyTrpLysGly
 140 150

TCCCCTGCCATCTTCCAGTCCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA
 SerProAlaIlePheGlnSerSerMetThrLysIleLeuGluProPheArgLysGlnAsnProAspIleValIleTyrGI
 160 170 180

GTACATGGCTGCCCCGTATGTGGCTCTGACCTGGAGATTGGGCAGCACAGGACCAAGATTGAGGAGCTGAGGCAGCACC
 nTyrMetAlaAlaLeuTyrValGlySerAspLeuGluIleGlyGlnHisArgThrLysIleGluGluLeuArgGlnHisL
 190 200 210

TGCTGAGGTGGGGCTGACCACCCCTGACAAGAAGCACCAGAAGGAGCCCCCTTCTGTGGATGGGCTATGAGCTGCAC
 euLeuArgTrpGlyLeuThrThrProAspLysLysHisGlnLysGluProProPheLeuTrpMetGlyTyrGluLeuHis
 220 230

CCGGACAAGTGGACTGTGCAGCCCATTTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG
 ProAspLysTrpThrValGlnProIleValLeuProGluLysAspSerTrpThrValAsnAspIleGlnLysLeuValGI
 240 250 260

CAAGCTGAAGTGGGCTCCCAAATCTACCCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCC
 yLysLeuAsnTrpAlaSerGlnIleTyrProGlyIleLysValArgGlnLeuCysLysLeuLeuArgGlyThrLysAlaI
 270 280 290

FIGURE 17A

TGACTGAGGTGATCCCCCTGACTGAGGAGGCTGAGCTGGAGCTGGCTGAGAACAGGGAGATCCTGAAGGAGCCTGTGCAT
 EüThrGluVolIleProLeuThrGluGluAlaGluLeuGluAlaGluAsnArgGluIleLeuLysGluProVolHis
 300 310

GGGGTGACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA
 GlyVolTyrTyrAspProSerLysAspLeuIleAlaGluIleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnIleTy
 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCACACCAATGATGTGAAGCAGCTGA
 rGlnGluProPheLysAsnLeuLysThrGlyLysTyrAlaArgMetArgGlyAlaHisThrAsnAspVolLysGlnLeuT
 350 360 370

CTGAGGCTGTGCAGAACATCACCAGTACTCCATTGTGATCTGGGGCAAGACCCCAAGTTCAAGCTGCCATCCAGAAG
 hrGluAlaVolGlnLysIleThrThrGluSerIleVolIleTrpGlyLysThrProLysPheLysLeuProIleGlnLys
 380 390

GAGACCTGGGAGACCTGGTGGACTGAGTACTGGCAGGCCACCTGGATCCCTGAGTGGGAGTTTGTGAACACCCCCCCT
 GluThrTrpGluThrTrpTrpThrGluTyrTrpGlnAlaThrTrpIleProGluTrpGluPheVolAsnThrProProLe
 400 410 420

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTTGTGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG
 uVolLysLeuTrpTyrGlnLeuGluLysGluProIleVolGlyAlaGluThrPheTyrValAlaGlyAlaAlaAsnArgG
 430 440 450

AGACCAAGCTGGGCAAGGCTGGCTATGTGACCAACAGGGGCAGGCAGAAGGTGGTGACCTGACTGACACCACCAACCAG
 luThrLysLeuGlyLysAlaGlyTyrVolThrAsnArgGlyArgGlnLysVolVolThrLeuThrAspThrThrAsnGln
 460 470

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCCTCCAGTATGC
 LysThrAlaLeuGlnAlaIleTyrLeuAlaLeuGlnAspSerGlyLeuGluVolAsnIleVolThrAlaSerGlnTyrAl
 480 490 500

CCTGGGCATCATCCAGGCCAGCCTGATCAGTCTGAGTCTGAGCTGGTGAACCAGATCATTGAGCAGCTGATCAAGAAGG
 aLeuGlyIleIleGlnAlaGlnProAspGlnSerGluSerGluLeuVolAsnGlnIleIleGluGlnLeuIleLysLysG
 510 520 530

AGAAGGTGTACCTGGCCTGGGTGCCTGCCACAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
 luLysVolTyrLeuAlaTrpVolProAlaHisLysGlyIleGlyGlyAsnGluGlnVolAspLysLeuVolSerAlaGly
 540 550

ATCAGGAAGCTGCTGTTCCCTGGATGGCATTGACAAGCCCCAGGATGACCATGAGAAGTACCACTCCAAGTGGAGGGCTAT
 IleArgLysVolLeuPheLeuAspGlyIleAspLysAlaGlnAspGluHisGluLysTyrHisSerAsnTrpArgAlaMe
 560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTCCAGCTGAAGGGGAGG
tAlaSerAspPheAsnLeuProProValVolAlaLysGluIleVolAlaSerCysAspLysCysGlnLeuLysGlyGluA
590 600 610

CCATGCATGGGCAGGTGGACTGCTCCCTGGCATCTGGCAGCTGGCCTGCACCCACCTGGAGGGCAAGGTGATCCTGGTG
lAlaMetHisGlyGlnVolAspCysSerProGlyIleTrpGlnLeuAlaCysThrHisLeuGluGlyLysVolIleLeuVol
620 630

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCTACTTCTGCT
AlaVolHisVolAlaSerGlyTyrIleGluAlaGluVolIleProAlaGluThrGlyGlnGluThrAlaTyrPheLeuLe
640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACCTTCACTGGGGCCACAGTGAGGGCTG
uLysLeuAlaGlyArgTrpProValLysThrIleHisThrAlaAsnGlySerAsnPheThrGlyAlaThrVolArgAlaA
670 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTGGCATCCCTACAACCCCCAGTCCAGGGGGTGGTGGCCTCCATGAAC
lAlaCysTrpTrpAlaGlyIleLysGlnGluPheGlyIleProTyrAsnProGlnSerGlnGlyVolVolAlaSerMetAsn
700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTTCA
LysGluLeuLysLysIleIleGlyGlnVolArgAspGlnAlaGluHisLeuLysThrAlaVolGlnMetAlaVolPheIle
720 730 740

CCACAACCTTCAAGAGGAAGGGGGCATCGGGGGCTACTCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlyIleGlyGlyTyrSerAlaGlyGluArgIleVolAspIleIleAlaThrAspIleG
750 760 770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAAGCTTCAAGGCTGACTACAGGAGCTCCAGGAACCCCTGTGG
lThrLysGluLeuGlnLysGlnIleThrLysIleGlnAsnPheArgVolTyrTyrArgAspSerArgAsnProLeuTrp
780 790

AAGGGCCCTGCCAAGCTGCTGTGAAGGGGAGGGGGCTGTGGTGATCCAGGACAACCTTGACATCAAGGTGGTGGCCAG
LysGlyProAlaLysLeuLeuTrpLysGlyGluGlyAlaVolVolIleGlnAspAsnSerAspIleLysVolVolProAr
800 810 820

GAGGAAGGCCAAGATCATCAGGGACTATGGCAAGCAGATGGCTGGGGATGACTGTGTGGCCTCCAGGCAGGATGAGGACT
gArgLysAlaLysIleIleArgAspTyrGlyLysGlnMetAlaGlyAspAspCysVolAlaSerArgGlnAspGluAspx
830 840 850

AAAGCCCGGGCAGATC" (SEQ ID NO: 3)
Xx BgllI (SEQ ID NO: 4)

FIGURE 17C

| | | |
|-----|---|------|
| WT | - ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT | -42 |
| | | |
| OPT | - ATG GGC GGC AAG TGG TCC AAG AGG TCC GTG CCC GGC TGG TCC | |
| | M G G K W S K R S V P G W S | -14 |
| WT | - ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT | -84 |
| | | |
| OPT | - ACC GTG AGG GAG AGG ATG AGG AGG GCC GAG CCC GCC GCC GAC | |
| | T V R E R M R R A E P A A D | -28 |
| WT | - AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA | -126 |
| | | |
| OPT | - AGG GTG AGG AGG ACC GAG CCC GCC GCC GTG GGC GTG GGC GCC | |
| | R V R R T E P A A V G V G A | -42 |
| WT | - GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC | -168 |
| | | |
| OPT | - GTG TCC AGG GAC CTG GAG AAG CAC GGC GCC ATC ACC TCC TCC | |
| | V S R D L E K H G A I T S S | -56 |
| WT | - AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA | -210 |
| | | |
| OPT | - AAC ACC GCC GCC ACC AAC GCC GAC TGC GCC TGG CTG GAG GCC | |
| | N T A A T N A D C A W L E A | -70 |
| WT | - CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA | -252 |
| | | |
| OPT | - CAG GAG GAC GAG GAG GTG GGC TTC CCC GTG AGG CCC CAG GTG | |
| | Q E D E E V G F P V R P Q V | -84 |
| WT | - CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC | -294 |
| | | |
| OPT | - CCC CTG AGG CCC ATG ACC TAC AAG GGC GCC GTG GAC CTG TCC | |
| | P L R P M T Y K G A V D L S | -98 |
| WT | - CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC | -336 |
| | | |
| OPT | - CAC TTC CTG AAG GAG AAG GGC GGC CTG GAG GGC CTG ATC CAC | |
| | H F L K E K G G L E G L I H | -112 |
| WT | - TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC | -378 |
| | | |
| OPT | - TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC | |
| | S Q K R Q D I L D L W V Y H | -126 |
| WT | - ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG | -420 |
| | | |
| OPT | - ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC | |
| | T Q G Y F P D W Q N Y T P G | -140 |

FIGURE 19A

| | | |
|-----|--|------|
| WT | - CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG | -462 |
| | | |
| OPT | - CCC GGC ATC AGG TTC CCC CTG ACC TTC GGC TGG TGC TTC AAG | |
| | P G I R F P L T F G W C F K | -154 |
| WT | - CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA | -504 |
| | | |
| OPT | - CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG | |
| | L V P V E P E K V E E A N E | -168 |
| WT | - GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG | -546 |
| | | |
| OPT | - GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC | |
| | G E N N C L L H P M S Q H G | -182 |
| WT | - ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC | -588 |
| | | |
| OPT | - ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC | |
| | I E D P E K E V L E W R F D | -196 |
| WT | - AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG | -630 |
| | | |
| OPT | - TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC | |
| | S K L A F H H V A R E L H P | -210 |
| WT | - GAG TAC TAC AAG GAC TGC TGA (SEQ ID NO:30) | -651 |
| | | |
| OPT | - GAG TAC TAC AAG GAC TGC TAA (contained within SEQ ID NO:9) | |
| | E Y Y K D C (SEQ ID NO:10) | -216 |

FIGURE 19B

V1Jns/nef

PstI *BglII*
CATGGGTCCTTTTCTGCAAGTCACCGTCCTTGAAGATCTGCCACC ATG GGC GGC ANG TGG TCC ANG AGG TCC GTG CCC
M G G K W S K R S V P

. CAC CCC GAG TAC TAC ANG GAC TGC TAA AGCCCGGAGATCTGCTGTGCTTCTAGTTGCCAGC (SEQ ID NO: 38)
H P E Y Y K D C * (contained within SEQ ID NO: 10)

SrfI *BglII*

V1Jns/nef(G2A.LLAA)

PstI *BglII*
CATGGGTCCTTTTCTGCAAGTCACCGTCCTTGAAGATCTGCCACC ATG GCC GGC ANG TGG TCC ANG AGG TCC GTG CCC
M A G K W S K R S V P

. CAC CCC GAG TAC TAC ANG GAC TGC TAA AGCCCGGAGATCTGCTGTGCTTCTAGTTGCCAGC (SEQ ID NO: 39)
H P E Y Y K D C * (contained within SEQ ID NO: 14)

SrfI *BglII*

V1Jns/tpanef & V1Jns/tpanef(LLAA)

PstI *BglII*
CATGGGTCCTTTTCTGCAAGTCACCGTCCTTGAATCTAGATCACC ATG GAT GCA ATG MAG AGA GGG CTC TGC TGT GTG
M D A M K R G L C C V

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG ALC ICC TCC ANG AGG TCC GTG CCC
L L L C G A V F V S P S E I S S K R S V P

BglII

. CAC CCC GAG TAC TAC ANG GAC TGC TAA AGCCCGGAGATCTGCTGTGCTTCTAGTTGCCAGC (SEQ ID NO: 40)
H P E Y Y K D C * (contained within SEQ ID NO: 16)

SrfI *BglII*

FIGURE 20

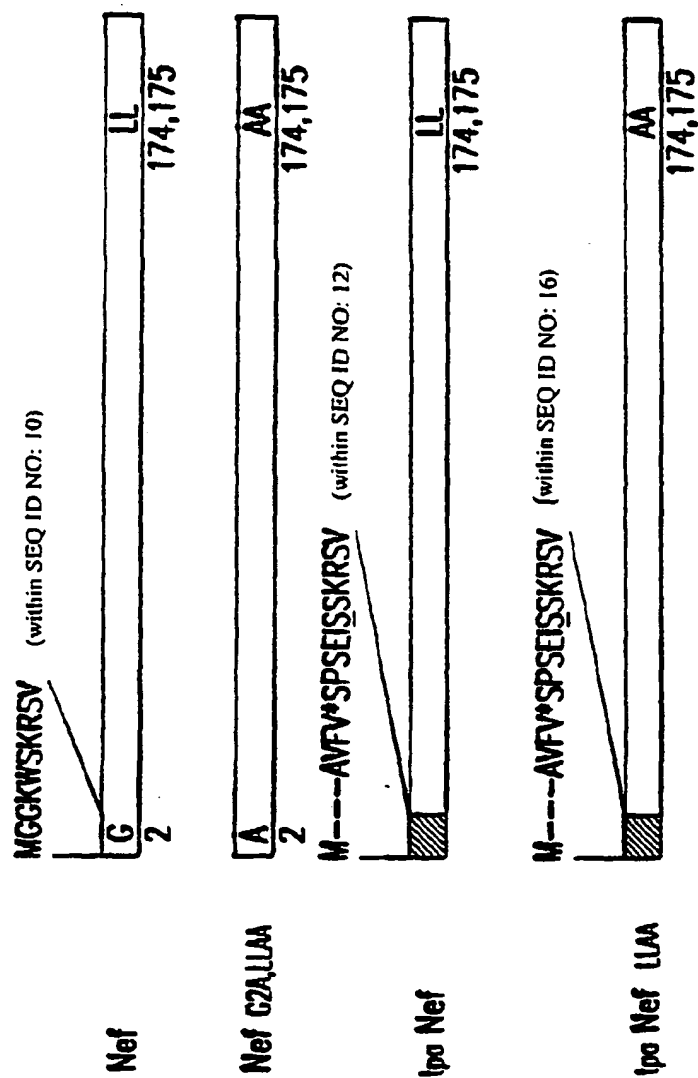


FIGURE 21

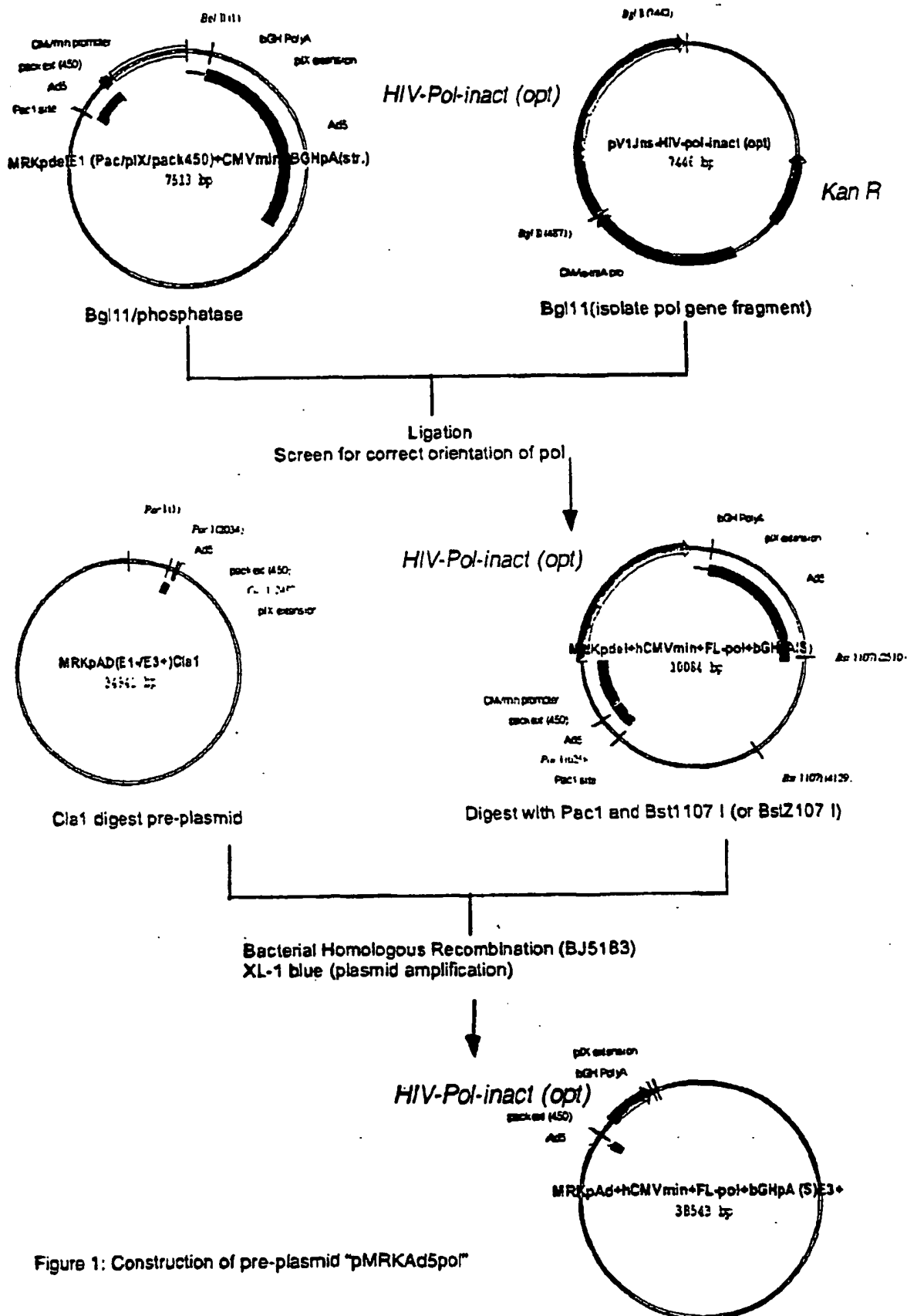


Figure 1: Construction of pre-plasmid "pMRKAd5pol"

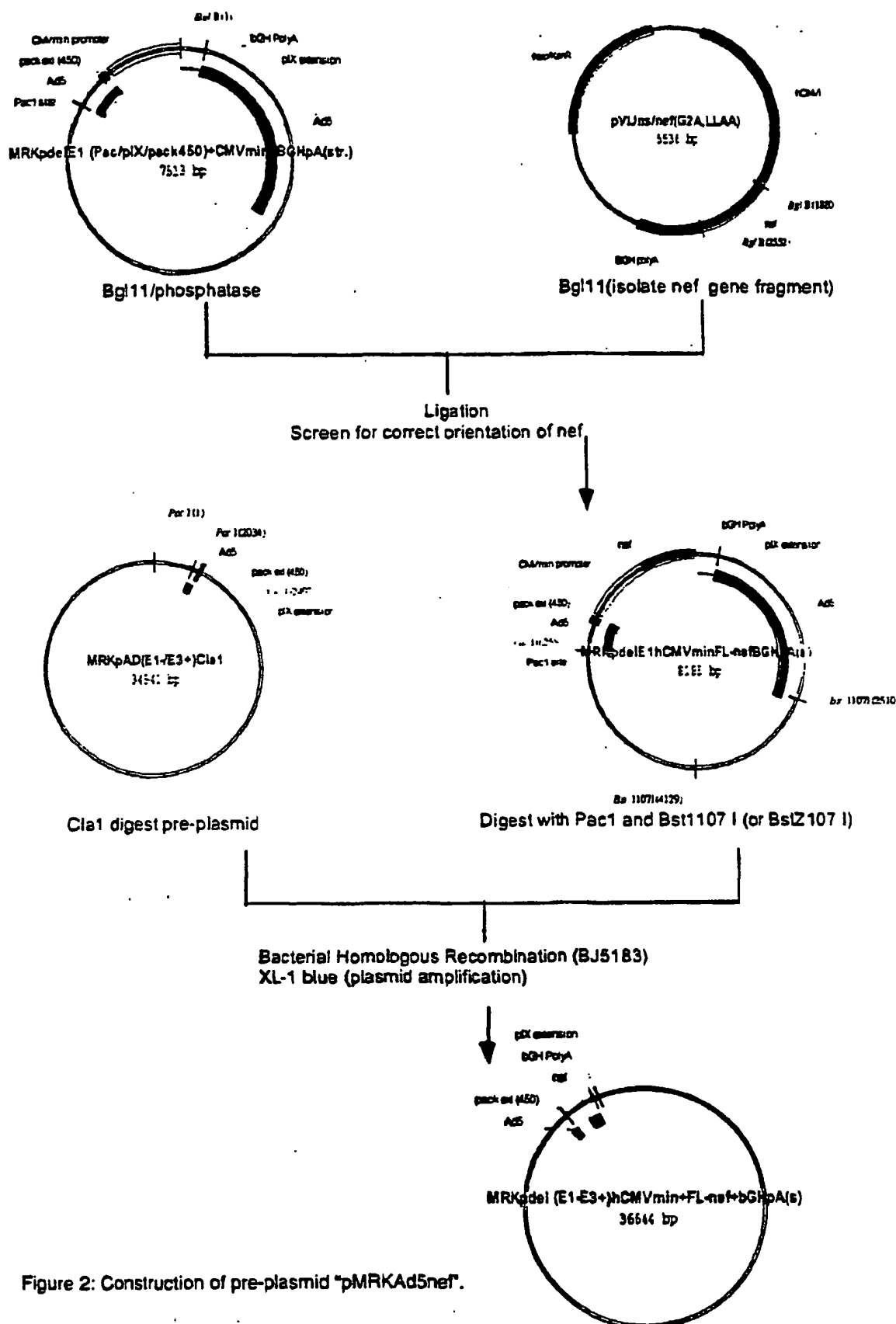
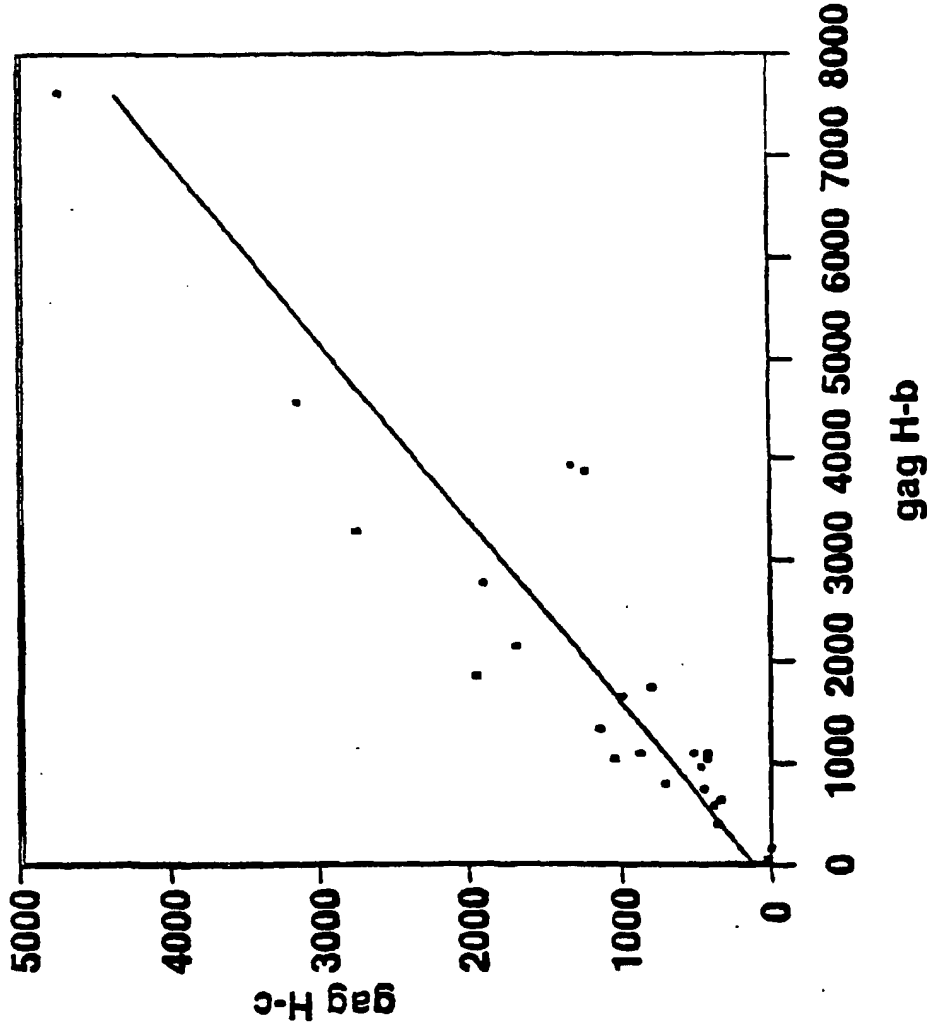


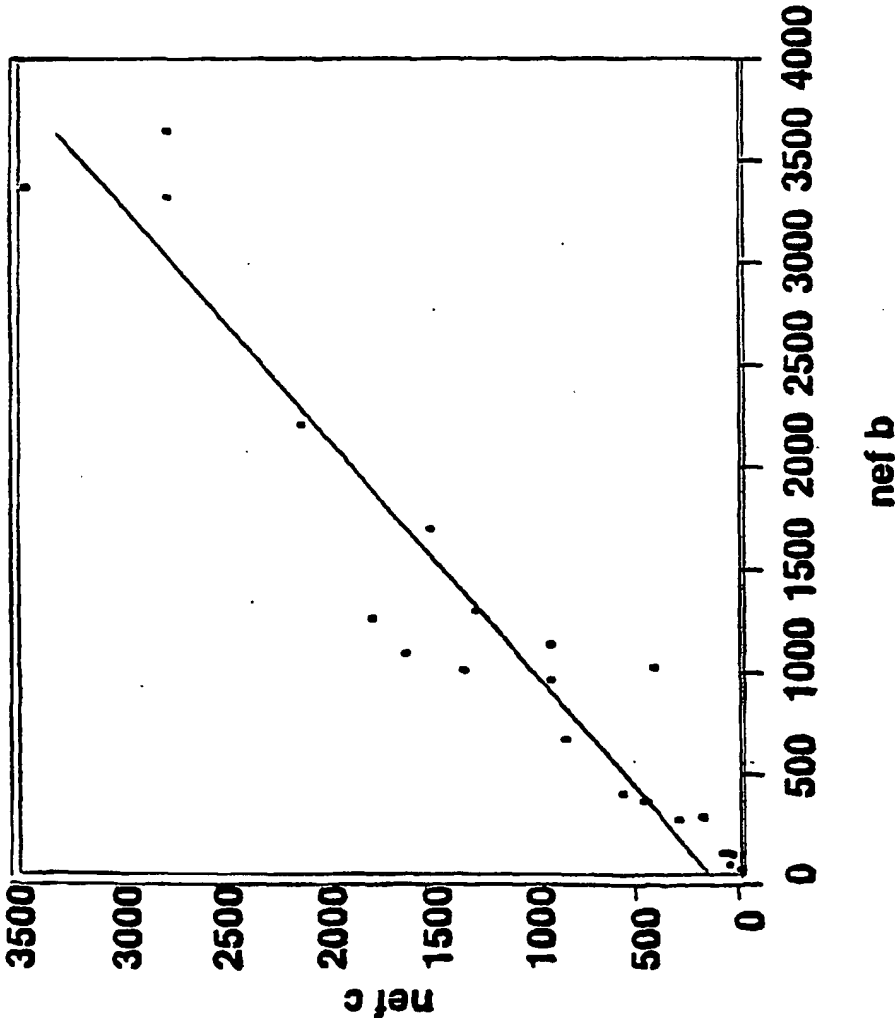
Figure 2: Construction of pre-plasmid "pMRKAd5nef".

Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects



| | |
|-------------------------------------|----------|
| Linear Fit | |
| gag H-c = 111.603 + 0.55866 gag H-b | |
| Summary of Fit | |
| RSquare | 0.816775 |
| RSquare Adj | 0.80914 |
| Root Mean Square Error | 474.9639 |
| Mean of Response | 1158.115 |
| Observations (or Sum Wgts) | 26 |

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects



Summary of Fit

| | |
|----------------------------|----------|
| RSquare | 0.91685 |
| RSquare Adj | 0.91289 |
| Root Mean Square Error | 289.7718 |
| Mean of Response | 1096.435 |
| Observations (or Sum Wgts) | 23 |

FIGURE 25

MRKAd5pol MER1062
(MRKAd5 Pre-Adenoviral Vector Containing the LA opt pol Coding Region)

```

1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACTTTAGA CTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCCGCGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGSTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGGTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTIAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTGCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACCGGG CGGACCGTAA TACGGGTCAT

851 CATGACCTTA TGGGACTTTC CTACTTGGCA GTACATCTAC GTATTAGTCA
   GTACTGGAAT ACCCTGAAAG GATGAACCGT CATGTAGATG CATAATCACT

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Figure 26A

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

1251 GAGATCTACC ATGGCCCCCA TCTCCCCCAT TGAGACTGTG CCTGTGAAGC
CTCTAGATGG TACCGGGGGT AGAGGGGGTA ACTCTGACAC GGACACTTCG

1301 TGAAGCCTGG CATGGATGGC CCCAAGGTGA AGCAGTGGCC CCTGACTGAG
ACTTCGGACC GTACCTACCG GGGTTCCACT TCGTCACCGG GGACTGACTC

1351 GAGAAGATCA AGGCCCTGGT GGAAATCTGC ACTGAGATGG AGAAGGAGGG
CTCTTCTAGT TCCGGGACCA CCTTTAGACG TGACTCTACC TCTTCCTCCC

1401 CAAATCTCC AAGATTGGCC CCGAGAACCC CTACAACACC CCTGTGTTTG
GTTTTAGAGG TTCTAACCGG GGCTCTTGGG GATGTTGTGG GGACACAAAC

1451 CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG
GGTAGTTCTT CTTCTGAGG TGGTTCACCT CCTTCGACCA CCTGAAGTCC

1501 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC
CTCGACTTGT TCTCCTGGGT CCTGAAGACC CTCCACGTCG ACCCGTAGGG

1551 CCACCCCGCT GGCCTGAAGA AGAAGAAGTC TGTGACTGTG CTGGCTGTGG
GGTGGGGCGA CCGGACTTCT TCTTCTTCAG ACACTGACAC GACCGACACC

1601 GGGATGCCTA CTTCTCTGTG CCCCTGGATG AGGACTTCAG GAAGTACACT
CCCTACGGAT GAAGAGACAC GGGGACCTAC TCCTGAAGTC CTTCATGTGA

1651 GCCTTCACCA TCCCCTCCAT CAACAATGAG ACCCCTGGCA TCAGGTACCA
CGGAAGTGGT AGGGGAGGTA GTTGTTACTC TGGGGACCGT AGTCCATGGT

1701 GTACAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC ATCTTCCAGT
CATGTTACAC GACGGGGTCC CGACCTTCCC GAGGGGACGG TAGAAGGTCA

1751 CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
GGAGGTA CTG GTTCTAGGAC CTCGGGAAGT CCTTCGTCTT GGGACTGTAA

1801 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT
CACTAGATGG TCATGTACCG ACGGGACATA CACCCGAGAC TGGACCTCTA

Figure 24B

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1901  GGGGCCTGAC  CACCCCTGAC  AAGAAGCACC  AGAAGGAGCC  CCCCTTCCTG
      CCCC GGACTG  GTGGGGACTG  TTCTTCGTGG  TCTTCCTCGG  GGGGAAGGAC

1951  TGGATGGGCT  ATGAGCTGCA  CCCC GACAAG  TGGACTGTGC  AGCCCATTGT
      ACCTACCCGA  TACTCGACGT  GGGGCTGTTC  ACCTGACACG  TCGGGTAACA

2001  GCTGCCTGAG  AAGGACTCCT  GGACTGTGAA  TGACATCCAG  AAGCTGGTGG
      CGACGGACTC  TTCCTGAGGA  CCTGACACTT  ACTGTAGGTC  TTCGACCACC

2051  GCAAGCTGAA  CTGGGCCTCC  CAAATCTACC  CTGGCATCAA  GGTGAGGCAG
      CGTTCGACTT  GACCCGGAGG  GTT TAGATGG  GACCGTAGTT  CCACTCCGTC

2101  CTGTGCAAGC  TGCTGAGGGG  CACCAAGGCC  CTGACTGAGG  TGATCCCCCT
      GACACGTTCTG  ACGACTCCCC  GTGGTTCCGG  GACTGACTCC  ACTAGGGGGA

2151  GACTGAGGAG  GCTGAGCTGG  AGCTGGCTGA  GAACAGGGAG  ATCCTGAAGG
      CTGACTCCTC  CGACTCGACC  TCGACCGACT  CTTGTCCCTC  TAGGACTTCC

2201  AGCCTGTGCA  TGGGGTGTAC  TATGACCCCT  CCAAGGACCT  GATTGCTGAG
      TCGGACACGT  ACCCCACATG  ATACTGGGGA  GGTTCCTGGA  CTAACGACTC

2251  ATCCAGAAGC  AGGGCCAGGG  CCAGTGGACC  TACC AAATCT  ACCAGGAGCC
      TAGGTCTTCG  TCCCGGTCCC  GGTCACTTGG  ATGGTTTAGA  TGGTCTCTCG

2301  CTTCAAGAAC  CTGAAGACTG  GCAAGTATGC  CAGGATGAGG  GGGGCCCACA
      GAAGTTCTTG  GACTTCTGAC  CGTTCATACG  GTCCTACTCC  CCCC GGGTGT

2351  CCAATGATGT  GAAGCAGCTG  ACTGAGGCTG  TGCAGAAGAT  CACCACTGAG
      GGTACTACAC  CTTCGTCGAC  TGACTCCGAC  ACGTCTTCTA  GTGGTGACTC

2401  TCCATTGTGA  TCTGGGGCAA  GACCCCCAAG  TTCAAGCTGC  CCATCCAGAA
      AGGTAAACACT  AGACCCCGTT  CTGGGGGGTTC  AAGTTCGACG  GGTAGGTCTT

2451  GGAGACCTGG  GAGACCTGGT  GGACTGAGTA  CTGGCAGGCC  ACCTGGATCC
      CCTCTGGACC  CTCTGGACCA  CCTGACTCAT  GACCGTCCGG  TGGACCTAGG

2501  CTGAGTGGGA  GTTTGTGAAC  ACCCCCCCCC  TGGTGAAGCT  GTGGTACCAG
      GACTCACCCCT  CAAACACTTG  TGGGGGGGGG  ACCACTTCGA  CACCATGGTC

2551  CTGGAGAAGG  AGCCCATTGT  GGGGGCTGAG  ACCTTCTATG  TGGCTGGGGC
      GACCTCTTCC  TCGGGTAACA  CCCCCGACTC  TGGAAGATAC  ACCGACCCCG

2601  TGCCAACAGG  GAGACCAAGC  TGGGCAAGGC  TGGCTATGTG  ACCAACAGGG
      ACGGTTGTCC  CTCTGGTTCTG  ACCCGTTCCG  ACCGATACAC  TGGTTGTCCC

2651  GCAGGCAGAA  GGTGGTGACC  CTGACTGACA  CCACCAACCA  GAAGACTGCC
      CGTCCGTCTT  CCACCACTGG  GACTGACTGT  GGTGGTTGGT  CTTCTGACGG

2701  CTCCAGGCCA  TCTACCTGGC  CCTCCAGGAC  TCTGGCCTGG  AGGTGAACAT
      GAGGTCCGCT  AGATGGACCG  GGAGGTCCTG  AGACCGGACC  TCCACTTGTA

2751  TGTGACTGCC  TCCCAGTATG  CCCTGGGCAT  CATCCAGGCC  CAGCCTGATC
      AACTGACGG  AGGGTCATAC  GGGACCCGTA  GTAGGTCCGG  GTCGGACTAG

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Figure 26 C

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2851  GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGGCAA
      CTCTTCCACA TGGACCGGAC CCACGGACGG GTGTTCCCGT AACCCCCGTT

2901  TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC
      ACTCGTCCAC CTGTTGACAC ACAGACGACC GTAGTCCTTC CACGACAAGG

2951  TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
      ACCTACCGTA ACTGTTCCGG GTCCTACTCG TACTCTTCAT GGTGAGGTTG

3001  TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA
      ACCTCCCGAT ACCGGAGACT GAAGTTGGAC GGGGGACACC ACCGATTCTT

3051  GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG
      CTAACACCGG AGGACACTGT TCACGGTCGA CTTCCCCCTC CGGTACGTAC

3101  GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG
      CCGTCCACCT GACGAGGGGA CCGTAGACCG TCGACCGGAC GTGGGTGGAC

3151  GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA
      CTCCCGTTCC ACTAGGACCA CCGACACGTA CACCGGAGGC CGATGTAAGT

3201  GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC
      CCGACTCCAC TAGGGACGAC TCTGTCCGGT CCTCTGACGG ATGAAGGACG

3251  TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
      ACTTCGACCG ACCGTCCACC GGACACTTCT GGTAGGTGTG ACGGTTACCG

3301  TCCAACCTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT
      AGGTTGAAGT GACCCCGGTG TCACTCCCGA CGGACGACCA CCCGACCGTA

3351  CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG
      GTTCGTCCTC AAACCGTAGG GGATGTTGGG GGTGAGGGTC CCCCACCACC

3401  CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG
      GGAGGTACTT GTTCCTCGAC TTCTTCTAGT AACCCGTCCA CTCCCTGGTC

3451  GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACCTT
      CGACTCGTGG ACTTCTGTG ACACGTCTAC CGACACAAGT AGGTGTTGAA

3501  CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG
      GTTCTCCTTC CCCCCGTAGC CCCCAGTAGG GCGACCCCTC TCCTAACACC

3551  ACATCATTCG CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
      TGTAATAACG GTGTCTGTAG GTCTGGTTCC TCGAGGTCTT CGTCTAGTGG

3601  AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG
      TTCTAGGTCT TGAAGTCCCA CATGATGTCC CTGAGGTCCT TGGGGGACAC

3651  GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC
      CTTCCCGGGA CGGTTCGACG ACACCTTCCC CCTCCCCCGA CACCACTAGG

3701  AGGACAACCTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC
      TCCTGTTGAG ACTGTAGTTC CACCACGGGT CCTCCTTCCG GTTCTAGTAG

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Figure 26 D

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3801  GGATGAGGAC TAAAGCCCGG GCAGATCTGC TGTGCCTTCT AGTTGCCAGC
      CCTACTCCTG ATTTCTGGGCC CGTCTAGACG ACACGGAAGA TCAACGGTCC

3851  CATCTGTTGT TTGCCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC
      GTAGACAACA AACGGGGAGG GGGCACGGAA GGAAGTGGGA CCTTCCACGG

3901  ACTCCCCTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTTGTCT
      TGAGGGTGAC AGGAAAGGAT TATTTTACTC CTTTAACGTA GCGTAACAGA

3951  GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG
      CTCATCCACA GTAAGATAAG ACCCCCCACC CCACCCCGTC CTGTCGTTC

4001  GGGAGGATTG GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT
      CCCTCCTAAC CCTTCTGTTA TCGTCCGTAC GACCCCTACG CCACCCGAGA

4051  ATGGCCGATC GCGCGCCGT ACTGAAATGT GTGGGCGTGG CTTAAGGGTG
      TACCGGCTAG CCGCGCGGCA TGACTTTACA CACCCGCACC GAATTCCCAC

4101  GGAAAGAATA TATAAGGTGG GGGTCTTATG TAGTTTTGTA TCTGTTTTGC
      CCTTCTTAT ATATTCCACC CCCAGAATAC ATCAAAACAT AGACAAAACG

4151  AGCAGCCGCC GCCGCCATGA GCACCAACTC GTTTGATGGA AGCATTGTGA
      TCGTCGGCGG CCGCGGTACT CGTGGTTGAG CAAACTACCT TCGTAACACT

4201  GCTCATATTT GACAACGCGC ATGCCCCCAT GGGCCGGGGT GCGTCAGAAT
      CGAGTATAAA CTGTTGCGCG TACGGGGGTA CCCGGCCCCA CGCAGTCTTA

4251  GTGATGGGCT CCAGCATTGA TGGTCGCCCC GTCCTGCCCC CAAACTCTAC
      CACTACCCGA GGTCGTAACT ACCAGCGGGG CAGGACGGGC GTTTGAGATG

4301  TACCTTGACC TACGAGACCG TGTCTGGAAC GCCGTTGGAG ACTGCAGCCT
      ATGGAAGTGG ATGCTCTGGC ACAGACCTTG CGGCAACCTC TGACGTCCGA

4351  CCGCCGCCGC TTCAGCCGCT GCAGCCACCG CCCGCGGGAT TGTGACTGAC
      GCGCGCGGCG AAGTCGGCGA CGTCGGTGGC GGGCGCCCTA ACACTGACTG

4401  TTTGCTTTCC TGAGCCCCTG TGCAAACAGT GCAGCTTCCC GTTCATCCGC
      AAACGAAAGG ACTCGGGCGA ACGTTTGTCA CGTCGAAGGG CAAGTAGGCG

4451  CCGCGATGAC AAGTTGACGG CTCTTTTGGC ACAATTGGAT TCTTTGACCC
      GCGGCTACTG TTCAACTGCC GAGAAAACCG TGTTAACCTA AGAAACTGGG

4501  GGGAAGTTAA TGTCGTTTCT CAGCAGCTGT TGGATCTGCG CCAGCAGGTT
      CCCTTGAATT ACAGCAAAGA GTCGTCGACA ACCTAGACGC GGTGCTCCAA

4551  TCTGCCCTGA AGGCTTCCTC CCCTCCCAAT GCGGTTTAAA ACATAAATAA
      AGACGGGACT TCCGAAGGAG GGGAGGGTTA CGCCAAATTT TGTATTTATT

4601  AAAACCAGAC TCTGTTTGA TTTGGATCAA GCAAGTGCTT TGCTGTCTTT
      TTTTGGTCTG AGACAAACCT AAACCTAGTT CGTTCACAGA ACGACAGAAA

4651  ATTTAGGGGT TTTGCGCGCG CGGTAGGCC GGGACCAGCG GTCTCGGTCTG
      TAAATCCCCA AAACGCGCGC GCCATCCGGG CCCTGGTCGC CAGAGCCAGC

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Figure 26E


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4751 GTTCAGATAC ATGGGCATAA GCCCGTCTCT GGGGTGGAGG TAGCACCCT
      CAAGTCTATG TACCCGTATT CGGGCAGAGA CCCCACCTCC ATCGTGGTGA

4801 GCAGAGCTTC ATGCTGCGGG GTGGTGTGTG AGATGATCCA GTCGTAGCAG
      CGTCTCGAAG TACGACGCCC CACCACAACA TCTACTAGGT CAGCATCGTC

4851 GAGCGCTGGG CGTGGTGCCT AAAAATGTCT TTCAGTAGCA AGCTGATTGC
      CTCGCGACCC GCACCACGGA TTTTACAGA AAGTCATCGT TCGACTAACG

4901 CAGGGGCAGG CCCTTGGTGT AAGTGTTTAC AAAGCGGTTA AGCTGGGATG
      GTCCCCGTCC GGGAAACCACA TTCACAAATG TTTCGCCAAT TCGACCCTAC

4951 GGTGCATACG TGGGGATATG AGATGCATCT TGGACTGTAT TTTTAGGTTG
      CCACGTATGC ACCCCTATAC TCTACGTAGA ACCTGACATA AAAATCCAAC

5001 GCTATGTTCC CAGCCATATC CCTCCGGGGA TTCATGTTGT GCAGAACCAC
      CGATACAAGG GTCGGTATAG GGAGGCCCTT AAGTACAACA CGTCTTGGTG

5051 CAGCACAGTG TATCCGGTGC ACTTGGGAAA TTTGTGTCATGT AGCTTAGAAG
      GTCGTGTCAC ATAGGCCACG TGAACCCTTT AAACAGTACA TCGAATCTTC

5101 GAAATGCGTG GAAGAACTTG GAGACGCCCT TGTGACCTCC AAGATTTTCC
      CTTTACGCAC CTTCTTGAAC CTCTGCGGGA ACACTGGAGG TTCTAAAAGG

5151 ATGCATTCTG CCATAATGAT GGCAATGGGC CCACGGGCGG CGGCCTGGGC
      TACGTAAGCA GGTATTACTA CCGTTACCCG GGTGCCCCGC GCCGGACCCG

5201 GAAGATATTT CTGGGATCAC TAACGTCATA GTTGTGTTCC AGGATGAGAT
      CTTCTATAAA GACCCTAGTG ATTGCAGTAT CAACACAAGG TCCTACTCTA

5251 CGTCATAGGC CATTTTTACA AAGCGCGGGC GGAGGGTGCC AGACTGCGGT
      GCAGTATCCG GTAAAAATGT TTCGCGCCCG CCTCCCACGG TCTGACGCCA

5301 ATAATGGTTC CATCCGGCCC AGGGGCGTAG TTACCCTCAC AGATTTGCAT
      TATTACCAAG GTAGGCCGGG TCCCCGCATC AATGGGAGTG TCTAAACGTA

5351 TTCCCACGCT TTGAGTTCAG ATGGGGGGAT CATGTCTACC TGCGGGGCGA
      AAGGGTGCGA AACTCAAGTC TACCCCTTA GTACAGATGG ACGCCCCGCT

5401 TGAAGAAAAC GGTTTCCGGG GTAGGGGAGA TCAGCTGGGA AGAAAGCAGG
      ACTTCTTTTG CCAAAGGCC CATCCCCTCT AGTCGACCCT TCTTTCGTCC

5451 TTCCTGAGCA GCTGCGACTT ACCGCAGCCG GTGGGCCCCT AAATCACACC
      AAGGACTCGT CGACGCTGAA TGGCGTCGGC CACCCGGGCA TTTAGTGTGG

5501 TATTACCGGC TGCAACTGGT AGTTAAGAGA GCTGCAGCTG CCGTCATCCC
      ATAATGGCCG ACGTTGACCA TCAATTCTCT CGACGTCGAC GGCAGTAGGG

5551 TGAGCAGGGG GGCCACTTCG TTAAGCATGT CCCTGACTCG CATGTTTTCC
      ACTCGTCCCC CCGGTGAAGC AATTCGTACA GGGACTGAGC GTACAAAAGG

5601 CTGACCAAAT CCGCCAGAAG GCGCTCGCCG CCCAGCGATA GCAGTTCTTG
      GACTGGTTTA GGCGGTCTTC CGCGAGCGGC GGGTCGCTAT CGTCAAGAAC

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Figure 26 F

5701 TTTTGAGCGT TTGACCAAGC AGTTCCAGGC GGTCCCACAG CTCGGTCACC
 AAAACTCGCA AACTGGTTCC TCAAGGTCCG CCAGGGTGTC GAGCCAGTGG

 5751 TGCTCTACGG CATCTCGATC CAGCATATCT CCTCGTTTCG CGGGTTGGGG
 ACGAGATGCC GTAGAGCTAG GTCGTATAGA GGAGCAAAGC GCCCAACCCC

 5801 CGGCTTTTCG TGTACGGCAG TAGTCGGTGC TCGTCCAGAC GGGCCAGGGT
 GCCGAAAGCG ACATGCCGTC ATCAGCCACG AGCAGGTCTG CCCGGTCCCA

 5851 CATGTCTTTC CACGGGCGCA GGGTCCTCGT CAGCGTAGTC TGGGTACAGG
 GTACAGAAAG GTGCCCCGCT CCCAGGAGCA GTCGCATCAG ACCCAGTGCC

 5901 TGAAGGGGTG CGCTCCGGGC TGC CGCTGG CCAGGGTGCG CTTGAGGCTG
 ACTTCCCCAC GCGAGGCCCG ACGCGCGACC GGTCCCACGC GAACTCCGAC

 5951 GTCCTGCTGG TGCTGAAGCG CTGCCGGTCT TCGCCCTGCG CGTCGGCCAG
 CAGGACGACC ACGACTTCGC GACGGCCAGA AGCGGGACGC GCAGCCGGTC

 6001 GTAGCATTTG ACCATGGTGT CATAGTCCAG CCCCTCCGCG GCGTGGCCCT
 CATCGTAAAC TGGTACCACA GTATCAGGTC GGGGAGGCGC CGCACC GGGA

 6051 TGGCGCGCAG CTTGCCCTTG GAGGAGGCGC CGCACGAGGG GCAGTGCAGA
 ACCGCGCGTC GAACGGGAAC CTCCTCCGCG GCGTGCTCCC CGTCACGTCT

 6101 CTTTTGAGGG CGTAGAGCTT GGGCGCGAGA AATACCGATT CCGGGGAGTA
 GAAAACTCCC GCATCTCGAA CCCGCGCTCT TTATGGCTAA GGCCCCCTCAT

 6151 GGCATCCGCG CCGCAGGCCC CGCAGACGGT CTCGCATTCC ACGAGCCAGG
 CCGTAGGCGC GGCGTCCGGG GCGTCTGCCA GAGCGTAAGG TGCTCGGTCC

 6201 TGAGCTCTGG CCGTTCGGGG TCAAAAACCA GGTTTCCCCC ATGCTTTTTG
 ACTCGAGACC GGCAAGCCCC AGTTTTTGGT CCAAAGGGGG TACGAAAAAC

 6251 ATGCGTTTCT TACCTCTGGT TTCCATGAGC CGGTGTCCAC GCTCGGTGAC
 TACGCAAAGA ATGGAGACCA AAGGTACTCG GCCACAGGTG CGAGCCACTG

 6301 GAAAAGGCTG TCCGTGTCCC CGTATACAGA CTTGAGAGGC CTGTCCTCGA
 CTTTTCCGAC AGGCACAGGG GCATATGTCT GAACTCTCCG GACAGGAGCT

 6351 GCGGTGTTCC GCGGTCTCC TCGTATAGAA ACTCGGACCA CTCTGAGACA
 CGCCACAAGG CGCCAGGAGG AGCATATCTT TGAGCCTGGT GAGACTCTGT

 6401 AAGGCTCGCG TCCAGGCCAG CACGAAGGAG GCTAAGTGGG AGGGGTAGCG
 TTCCGAGCGC AGGTCCGGTC GTGCTTCCTC CGATTACCC TCCCCATCGC

 6451 GTCGTTGTCC ACTAGGGGGT CCACTCGCTC CAGGGTGTGA AGACACATGT
 CAGCAACAGG TGATCCCCCA GGTGAGCGAG GTCCCACACT TCTGTGTACA

 6501 CGCCCTCTTC GGCATCAAGG AAGGTGATTG GTTTGTAGGT GTAGGCCACG
 GCGGGAGAAG CCGTAGTTCC TTCCACTAAC CAAACATCCA CATCCGGTGC

 6551 TGACCGGGTG TTCTGAAGG GGGGCTATAA AAGGGGGTGG GGGCGCGTTC
 ACTGGCCAC AAGGACTTCC CCCCAGATATT TTCCCCACC CCCGCGCAAG

Figure 266

6651 AGTACTCCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAGTT
TCATGAGGGA GACTTTTTCGC CCGTACTGAA GACGCGATTG TAACAGTCAA

6701 TCCAAAAACG AGGAGGATTT GATATTCACC TGGCECGCGG TGATGCCTTT
AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA

6751 GAGGGTGGCC GCATCCATCT GGTCAGAAAA GACAATCTTT TTGTTGTCAA
CTCCCAACCG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACACAGTT

6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG
CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC

6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT
CTCGCGTCCC AAACCAAAAA CAGCGCTAGC CGCGCGAGGA ACCGGCGCTA

6901 GTTTAGCTGC ACGTATTCGC GCGCAACGCA CCGCCATTCT GGAAAGACGG
CAAATCGACG TGCATAAGCG CGCGTTGCGT GCGGGTAAGC CCTTCTGCG

6951 TGGTGCCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG
ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTGGCGC CAACACGTCC

7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT
CACTGTTCCA GTTGCGACCA CCGATGGAGA GGCGCATCCG CGAGCAACCA

7051 CCAGCAGAGG CGGCCGCCCT TGCGCGAGCA GAATGGCGGT AGGGGGTCTA
GGTCGTCTCC GCCGGCGGGA ACGCGCTCGT CTTACCGCCA TCCCCAGAT

7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC
CGACGCAGAG CAGGCCCCC AGACGCAGGT GCCATTTCTG GGGCCGCTCG

7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CCTTGCAAGT CTAGCGCCTG
TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC

7201 CTGCCATGCG CGGGCGGCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC
GACGGTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCTG

7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTCTG
GGGTACCGTA CCCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC

7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT
ATTTGCATCT CCCCAGAGAG CTCATAAGGT TCTATACATC CCATCGTAGA

7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTCTG TGCGAGGGAG
AGGTGGCGCC TACGACCGCG CGTGCAATTAG CATATCAAGC ACGCTCCCTC

7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG
GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC

7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG
TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC

7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG
CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

Figure 26 H

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7601  TCTAGGGCGC AGTAGTCCAG GGTTCCTTG ATGATGTCAT ACTTATCCTG
      AGATCCCGCG TCATCAGGTC CCAAAGGAAC TACTACAGTA TGAATAGGAC

7651  TCCCTTTTTT TTCCACAGCT CGCGGTTGAG GACAACTCT TCGCGGTCTT
      AGGGAAAAAA AAGGTGTCGA GCGCCAAC TC GTTTTGAGA AGCGCCAGAA

7701  TCCAGTACTC TTGGATCGGA AACCCGTCGG CCTCCGAACG GTAAGAGCCT
      AGGTCATGAG AACCTAGCCT TTGGGCAGCC GGAGGCTTGC CATTCCTCGGA

7751  AGCATGTAGA ACTGGTTGAC GGCCTGGTAG GCGCAGCATC CCTTTTCTAC
      TCGTACATCT TGACCAACTG CCGGACCATC CGCGTCGTAG GGAAAAGATG

7801  GGGTAGCGCG TATGCCTGCG CGGCCTTCCG GAGCGAGGTG TGGGTGAGCG
      CCCATCGCGC ATACGGACGC GCCGGAAGGC CTCGCTCCAC ACCCACTCGC

7851  CAAAGGTGTC CCTGACCATG ACTTTGAGGT ACTGGTATTT GAAGTCAGTG
      GTTTCACAG GGA CTGGTAC TGAACTCCA TGACCATAAA CTTCACTCAC

7901  TCGTCGCATC CGCCCTGCTC CCAGAGCAAA AAGTCCGTGC GCTTTTGGGA
      AGCAGCGTAG GCGGGACGAG GGTCTCGTTT TTCAGGCACG CGAAAACTT

7951  ACGCGGATTT GGCAGGGCGA AGGTGACATC GTTGAAGAGT ATCTTTCCCG
      TGCGCCTAAA CCGTCCCGCT TCCACTGTAG CAACTTCTCA TAGAAAGGGC

8001  CGCGAGGCAT AAAGTTGCGT GTGATGCGGA AGGGTCCCGG CACCTCGGAA
      GCGCTCCGTA TTCAACGCA CACTACGCC TCCCAGGGCC GTGGAGCCTT

8051  CGGTTGTTAA TTACCTGGGC GGCAGCACG ATCTCGTCAA AGCCGTTGAT
      GCCAACAAAT AATGGACCCG CCGCTCGTGC TAGAGCAGTT TCGGCAACTA

8101  GTTGTGGCCC ACAATGTAAA GTTCCAAGAA GCGCGGGATG CCCTTGATGG
      CAACACCGGG TGTTACATTT CAAGGTTCTT CGCGCCCTAC GGGAACTACC

8151  AAGGCAATTT TTTAAGTTCC TCGTAGGTGA GCTCTTCAGG GGAGCTGAGC
      TTCCGTTAAA AAATTCAAGG AGCATCCACT CGAGAAGTCC CCTCGACTCG

8201  CCGTGCTCTG AAAGGGCCCA GTCTGCAAGA TGAGGGTTGG AAGCGACGAA
      GGCACGAGAC TTTCCTGGGT CAGACGTTCT ACTCCCAACC TTCGCTGCTT

8251  TGAGCTCCAC AGGTCACGGG CCATTAGCAT TTGCAGGTGG TCGCGAAAGG
      ACTCGAGGTG TCCAGTGCCC GGTAATCGTA AACGTCCACC AGCGCTTTCC

8301  TCCTAAACTG GCGACCTATG GCCATTTTTT CTGGGGTGAT GCAGTAGAAG
      AGGATTTGAC CGCTGGATAC CGGTAAAAAA GACCCCACTA CGTCATCTTC

8351  GTAAGCGGGT CTTGTTCCCA GCGGTCCCAT CCAAGGTTCTG CGGCTAGGTC
      CATTCGCCCA GAACAAGGGT CGCCAGGGTA GGTCCAAGC GCCGATCCAG

8401  TCGCGCGGCA GTCAC TAGAG GCTCATCTCC GCCGAAC TTC ATGACCAGCA
      AGCGCGCCGT CAGTGATCTC CGAGTAGAGG CGGCTTGAAG TACTGGTCGT

8451  TGAAGGGCAC GAGCTGCTTC CCAAAGGCC CCATCCAAGT ATAGGTCTCT
      ACTTCCCGTG CTCGACGAAG GGTTCCTGGG GGTAGGTTCA TATCCAGAGA

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Figure 26 I

8551 GAAGAACTGG ATCTCCCGCC ACCAATTGGA GGAGTGGCTA TTGATGTGGT
 CTTCTTGACC TAGAGGGCGG TGGTTAACCT CCTCACCGAT AACTACACCA

 8601 GAAAGTAGAA GTCCCTGCGA CGGGCCGAAC ACTCGTGCTG GCTTTTGTA
 CTTTCATCTT CAGGGACGCT GCCCGGCTTG TGAGCACGAC CGAAAACATT

 8651 AAACGTGCGC AGTACTGGCA GCGGTGCACG GGCTGTACAT CCTGCACGAG
 TTTGCACGCG TCATGACCGT CGCCACGTGC CCGACATGTA GGACGTGCTC

 8701 GTTGACCTGA CGACCGCGCA CAAGGAAGCA GAGTGGGAAT TTGAGCCCCT
 CAACTGGACT GCTGGCGCGT GTTCCTTCGT CTCACCCTTA AACTCGGGGA

 8751 CGCCTGGCGG GTTTGGCTGG TGGTCTTCTA CTTCCGGCTGC TTGTCCTTGA
 GCGGACCGCC CAAACCGACC ACCAGAAGAT GAAGCCGACG AACAGGAACT

 8801 CCGTCTGGCT GCTCGAGGGG AGTTACGGTG GATCGGACCA CCACGCCGCG
 GGCAGACCGA CGAGCTCCCC TCAATGCCAC CTAGCCTGGT GGTGCGGCGC

 8851 CGAGCCCAAA GTCCAGATGT CCGCGCGCGG CGGTCGGAGC TTGATGACAA
 GCTCGGGTTT CAGGTCTACA GCGCGCGCGC GCCAGCCTCG AACTACTGTT

 8901 CATCGCGCAG ATGGGAGCTG TCCATGGTCT GGAGCTCCCG CGGCGTCAGG
 GTAGCGCGTC TACCTCGAC AGGTACCAGA CCTCGAGGGC GCCGCAGTCC

 8951 TCAGGCGGGA GCTCCTGCAG GTTTACCTCG CATAGACGGG TCAGGGCGCG
 AGTCCGCCCT CGAGGACGTC CAAATGGAGC GTATCTGCCC AGTCCCGCGC

 9001 GGCTAGATCC AGGTGATACC TAATTTCCAG GGGCTGGTTG GTGGCGGCGT
 CCGATCTAGG TCCACTATGG ATTAAAGGTC CCCGACCAAC CACCGCCGCA

 9051 CGATGGCTTG CAAGAGGCCG CATCCCCGCG GCGCGACTAC GGTACCGCGC
 GCTACCGAAC GTTCTCCGGC GTAGGGGCGC CGCGCTGATG CCATGGCGCG

 9101 GGCGGGCGGT GGGCCGCGGG GGTGTCCTTG GATGATGCAT CTAAAAGCGG
 CCGCCCGCCA CCGGGCGCCC CCACAGGAAC CTACTACGTA GATTTTCGCC

 9151 TGACGCGGGC GAGCCCCCGG AGGTAGGGGG GGCTCCGGAC CCGCCGGGAG
 ACTGCGCCCG CTCGGGGGCC TCCATCCCCC CCGAGGCCTG GCGGGCCCTC

 9201 AGGGGGCAGG GGCACGTCGG CGCCGCGCGC GGGCAGGAGC TGGTGCTGCG
 TCCCCGTCC CCGTGCAGCC GCGGCGCGCG CCCGTCCTCG ACCACGACGC

 9251 CGCGTAGGTT GCTGGCGAAC GCGACGACGC GGCGGTTGAT CTCCTGAATC
 GCGCATCCAA CGACCGCTTG CGCTGCTGCG CCGCCAATA GAGGACTTAG

 9301 TGGCGCCTCT GCGTGAAGAC GACGGGCCCC GTGAGCTTGA ACCTGAAAGA
 ACCGCGGAGA CGCACTTCTG CTGCCCGGGC CACTCGAACT TGGACTTTCT

 9351 GAGTTTCGACA GAATCAATTT CGGTGTCGTT GACGGCGGGC TGGCGCAAAA
 CTCAAGCTGT CTTAGTTAAA GCCACAGCAA CTGCCGCGG ACCGCGTTTT

 9401 TCTCCTGCAC GTCTCCTGAG TTGTCTTGAT AGGCGATCTC GGCCATGAAC
 AGAGGACGTG CAGAGGACTC AACAGAACTA TCCGCTAGAG CCGGTACTTG

Figure 26 J

9501 GCGGCGGAGG TCGTTGGAAA TCGGGGCCAT GAGCTGCGAG AAGGCGTTGA
CCGCCGCTCC AGCAACCTTT ACGCCCGGTA CTCGACGCTC TTCCGCAACT

9551 GGCCTCCCTC GTTCCAGACG CGGCTGTAGA CCACGCCCCC TTCGGCATCG
CCGGAGGGAG CAAGGTCTGC GCCGACATCT GGTGCGGGGG AAGCCGTAGC

9601 CGGGCGCGCA TGACCACCTG CGCGAGATTG AGCTCCACGT GCCGGGCGAA
GCCCCGCGCT ACTGGTGGAC GCGCTCTAAC TCGAGGTGCA CGGCCCCGCTT

9651 GACGGCGTAG TTTCGCAGGC GCTGAAAGAG GTAGTTGAGG GTGGTGGCGG
CTGCCGCATC AAAGCGTCCG CGACTTTCTC CATCAACTCC CACCACCGCC

9701 TGTGTTCTGC CACGAAGAAG TACATAACCC AGCGTCGCAA CGTGGATTCTG
ACACAAGACG GTGCTTCTTC ATGTATTGGG TCGCAGCGTT GCACCTAAGC

9751 TTGATATCCC CCAAGGCCTC AAGGCGCTCC ATGGCCTCGT AGAAGTCCAC
AACTATAGGG GGTTCGAG GGTTCGCGAGG TACCGGAGCA TCTTCAGGTG

9801 GGCGAAGTTG AAAAAGTGGG AGTTGCGCGC CGACACGGTT AACTCCTCCT
CCGCTTCAAC TTTTGTGACC TCAACGCGCG GCTGTGCCAA TTGAGGAGGA

9851 CCAGAAGACG GATGAGCTCG GCGACAGTGT CGCGCACCTC GCGCTCAAAG
GGTCTTCTGC CTAATCGAGC CGCTGTCACA GCGCGTGGAG CGCGAGTTTC

9901 GCTACAGGGG CCTCTTCTTC TTCTTCAATC TCCTCTTCCA TAAGGGCCTC
CGATGTCCCC GGAGAAGAAG AAGAAGTTAG AGGAGAAGGT ATTCCCGGAG

9951 CCCTTCTTCT TCTTCTGGCG GCGGTGGGGG AGGGGGGACA CGGCGGCGAC
GGGAAGAAGA AGAAGACCGC CGCCACCCCC TCCCCCTGT GCCGCCGCTG

10001 GACGGCGCAC CGGGAGGCGG TCGACAAAGC GCTCGATCAT CTCCCCGCGG
CTGCCGCGTG GCCCTCCGCC AGCTGTTTCG CGAGCTAGTA GAGGGGCGCC

10051 CGACGGCGCA TGGTCTCGGT GACGGCGCGG CCGTTCTCGC GGGGGCGCAG
GCTGCCGCGT ACCAGAGCCA CTGCCGCGCC GGCAAGAGCG CCCCCGCGTC

10101 TTGGAAGACG CCGCCCGTCA TGTCCCGGTT ATGGGTGGC GGGGGGCTGC
AACCTTCTGC GCGGGGCACT ACAGGGCCAA TACCCAACCG CCCCCGACG

10151 CATGCGGCAG GGATACGGCG CTAACGATGC ATCTCAACAA TTGTTGTGTA
GTACGCCGTC CCTATGCCGC GATTGCTACG TAGAGTTGTT AACAACACAT

10201 GGTACTCCGC CGCCGAGGGA CCTGAGCGAG TCCGCATCGA CCGGATCGGA
CCATGAGGCG GCGGCTCCCT GGACTCGCTC AGGCGTAGCT GGCCTAGCCT

10251 AAACCTCTCG AGAAAGGCGT CTAACGATC ACAGTCGCAA GGTAGGCTGA
TTTGGAGAGC TCTTCCGCA GATTGGTCAG TGTGAGCGTT CCATCCGACT

10301 GCACCGTGGC GGGCGGCAGC GGGCGGCGGT CGGGGTGTGT TCTGGCGGAG
CGTGGCACCG CCCGCCGTCG CCCGCCGCCA GCCCCAACAA AGACCGCCTC

10351 GTGCTGCTGA TGATGTAATT AAAGTAGGCG GTCTTGAGAC GCGGATGGT
CACGACGACT ACTACATTAA TTTATCCGC CAGAACTCTG CCGCCTACCA

Figure 26 K

10451 CGGCCATGCC CCAGGCTTCG TTTTGACATC GGCGCAGGTC TTTGTTAGTAG
 GCCGGTACGG GGTCCGAAGC AAAACTGTAG CCGCGTCCAG AAACATCATC
 10501 TCTTGATGA GCCTTTCTAC CGGCACTTCT TCTTCTCCTT CCTCTTGTCC
 AGAACGTACT CGGAAAGATG GCCGTGAAGA AGAAGAGGAA GGAGAACAGG
 10551 TGCATCTCTT GCATCTATCG CTGCGGCGGC GGCGGAGTTT GGCCGTAGGT
 ACGTAGAGAA CGTAGATAGC GACGCCGCCG CCGCCTCAA CCGGCATCCA
 10601 GGCGCCCTCT TCCTCCCATG CGTGTGACCC CGAAGCCCCT CATCGGCTGA
 CCGCGGGAGA AGGAGGGTAC GCACACTGGG GCTTCGGGGA GTAGCCGACT
 10651 AGCAGGGCTA GGTGCGCGAC AACGCGCTCG GCTAATATGG CCTGCTGCAC
 TCGTCCCGAT CCAGCCGCTG TTGCGCGAGC CGATTATACC GGACGACGTG
 10701 CTGCGTGAGG GTAGACTGGA AGTCATCCAT GTCCACAAAG CCGTGGTATG
 GACGCACTCC CATCTGACCT TCAGTAGGTA CAGGTGTTTC GCCACCATAC
 10751 CGCCCGTGTT GATGGTGTA GTGCAGTTGG CCATAACGGA CCAGTTAAGC
 GCGGGCAGAA CTACCACATT CACGTCAACC GGTATTGCCT GGTCAATTGC
 10801 GTCTGGTGAC CCGGCTGCGA GAGCTCGGTG TACCTGAGAC GCGAGTAAGC
 CAGACCACTG GGCCGACGCT CTCGAGCCAC ATGGACTCTG CGCTCATTCG
 10851 CCTCGAGTCA AATACGTAGT CGTTGCAAGT CCGCACCAGG TACTGGTATC
 GGAGCTCAGT TTATGCATCA GCAACGTTCA GGCGTGGTCC ATGACCATAG
 10901 CCACCAAAAA GTGCGGCGGC GGCTGGCGGT AGAGGGGCCA GCGTAGGGTG
 GGTGGTTTTT CACGCCGCCG CCGACCGCCA TCTCCCCGGT CGCATCCAC
 10951 GCCGGGGCTC CGGGGGCGAG ATCTTCCAAC ATAAGGCGAT GATATCCGTA
 CGGCCCGAG GCCCGCGCTC TAGAAGGTTG TATTCCGCTA CTATAGGCAT
 11001 GATGTACCTG GACATCCAGG TGATGCCGGC GGCGGTGGTG GAGGCGCGCG
 CTACATGGAC CTGTAGGTCC ACTACGGCCG CCGCCACCAC CTCCGCGCGC
 11051 GAAAGTCGCG GACGCGGTTC CAGATGTTGC GCAGCGGCAA AAAGTGCTCC
 CTTTCAGCGC CTGCGCCAAG GTCTACAACG CGTCGCCGTT TTTACGAGG
 11101 ATGGTCGGGA CGCTCTGGCC GGTGAGGCGC GCGCAATCGT TGACGCTCTA
 TACCAGCCCT GCGAGACCGG CCAGTCCGCG CGCGTTAGCA ACTGCGAGAT
 11151 GACCGTGCAA AAGGAGAGCC TGTAAGCGGG CACTCTTCCG TGGTCTGGTG
 CTGGCACGTT TTCTCTCGG ACATTGCCCC GTGAGAAGGC ACCAGACCAC
 11201 GATAAATTCG CAAGGGTATC ATGGCGGACG ACCGGGGTTC GAGCCCCGTA
 CTATTTAAGC GTTCCCATAG TACCGCCTGC TGGCCCCAAG CTCGGGGCAT
 11251 TCCGGCCGTC CGCCGTGATC CATGCGGTTA CCGCCCGCGT GTCGAACCCA
 AGGCCGCGAG GCGGCACTAG GTACGCCAAT GGCGGGCGCA CAGCTTGGGT
 11301 GGTGTGCGAC GTCAGACAAC GGGGGAGTGC TCCTTTTGGC TTCCTTCCAG
 CCACACGCTG CAGTCTGTTG CCCCCTCACG AGGAAAACCG AAGGAAGGTC

Figure 26L

11401 AAGCGGTTAG GCTGGAAAGC GAAAGCATT AAGTGGCTCGC TCCCTGTAGC
TTCGCCAATC CGACCTTTTCG CTTTCGTAAT TCACCGAGCG AGGGACATCG

11451 CGGAGGGTTA TTTTCCAAGG GTTGAGTCGC GGGACCCCCG GTTCGAGTCT
GCCTCCCAAT AAAAGGTTCC CAACTCAGCG CCCTGGGGGC CAAGCTCAGA

11501 CGGACCGGCC GGA CTGCGGC GAACGGGGGT TTGCCTCCCC GTCATGCAAG
GCCTGGCCGG CCTGACGCCG CTTGCCCCCA AACGGAGGGG CAGTACGTTT

11551 ACCCCGCTTG CAAATTCTCT CGGAAACAGG GACGAGCCCC TTTTGTGTT
TGGGGCGAAC GTTTAAGGAG GCCTTTGTCC CTGCTCGGGG AAAAAACGAA

11601 TTCCAGATG CATCCGGTGC TGC GGCAGAT GCGCCCCCT CCTCAGCAGC
AAGGGTCTAC GTAGGCCACG ACGCCGTCTA CGCGGGGGGA GGAGTCGTCTG

11651 GGCAAGAGCA AGAGCAGCGG CAGACATGCA GGGCACCCCTC CCCTCCTCCT
CCGTTCTCGT TCTCGTCGCC GTCTGTACGT CCCGTGGGAG GGGAGGAGGA

11701 ACCGCGTCAG GAGGGGCGAC ATCCGCGGTT GACGCGGCAG CAGATGGTGA
TGGCGCAGTC CTCCCCGCTG TAGGCGCCAA CTGCGCCGTC GTCTACCACT

11751 TTACGAACCC CCGCGGCGCC GGGCCCCGCA CTACCTGGAC TTGGAGGAGG
AATGCTTGGG GGCGCCGCGG CCCGGGCCGT GATGGACCTG AACCTCCTCC

11801 GCGAGGGCCT GGCGCGGCTA GGAGCGCCCT CTCCTGAGCG GCACCCAAGG
CGCTCCCGGA CCGCGCCGAT CCTCGCGGGA GAGGACTCGC CGTGGGTTC

11851 GTGCAGCTGA AGCGTGATAC GCGTGAGGCG TACGTGCCGC GGCAGAACCT
CACGTGCACT TCGCACTATG CGCACTCCGC ATGCACGGCG CCGTCTTGGA

11901 GTTTCGCGAC CGCGAGGGAG AGGAGCCCGA GGAGATGCGG GATCGAAAGT
CAAAGCGCTG GCGCTCCCTC TCCTCGGGCT CCTCTACGCC CTAGCTTTCA

11951 TCCACGCAGG GCGCGAGCTG CGGCATGGCC TGAATCGCGA GCGGTTGCTG
AGGTGCGTCC CGCGCTCGAC GCCGTACCGG ACTTAGCGCT CGCCAACGAC

12001 CGCGAGGAGG ACTTTGAGCC CGACGCGCGA ACCGGGATTA GTCCCGCGCG
GCGCTCCTCC TGAAACTCGG GCTGCGCGCT TGGCCCTAAT CAGGGCGCGC

12051 CGCACACGTG GCGGCCGCCG ACCTGGTAAC CGCATACGAG CAGACGGTGA
GCGTGTGCAC CGCCGGCGGC TGGACCATTG GCGTATGCTC GTCTGCCACT

12101 ACCAGGAGAT TAACTTTCAA AAAAGCTTTA ACAACCACGT GCGTACGCTT
TGGTCTCTA ATTGAAAGTT TTTTCGAAAT TGTTGGTGCA CGCATGCGAA

12151 GTGGCGCGCG AGGAGGTGGC TATAGGACTG ATGCATCTGT GGGACTTTGT
CACCGCGCGC TCCTCCACCG ATATCCTGAC TACGTAGACA CCCTGAAACA

12201 AAGCGCGCTG GAGCAAAACC CAAATAGCAA GCCGCTCATG GCGCAGCTGT
TTCGCGCGAC CTCGTTTTGG GTTTATCGTT CGGCGAGTAC CGCGTCGACA

12251 TCCTTATAGT GCAGCACAGC AGGGACAACG AGGCATTGAG GGATGCGCTG
AGGAATATCA CGTCGTGTCTG TCCCTGTTGC TCCGTAAGTC CCTACGCGAC

Figure 26 M

12351 CCTGCAGAGC ATAGTGGTGC AGGAGCGCAG CTTGAGCCTG GCTGACAAGG
 GGACGTCTCG TATCACCACG TCCTCGCGTC GAACTCGGAC CGACTGTTCC
 12401 TGGCCGCCAT CAACTATTCC ATGCTTAGCC TGGGCAAGTT TTACGCCCCG
 ACCGGCGGTA GTTGATAAGG TACGAATCGG ACCCGTTCAA AATGCGGGCG
 12451 AAGATATAACC ATACCCCTTA CGTTCCCATG GACAAGGAGG TAAAGATCGA
 TTCTATATGG TATGGGGAAT GCAAGGTAT CTGTTCTCTC ATTTCTAGCT
 12501 GGGGTCTTAC ATGCGCATGG CGCTGAAGGT GCTTACCTTG AGCGACGACC
 CCCCAGATG TACGCGTACC GCGACTTCCA CGAATGGAAC TCGCTGCTGG
 12551 TGGGCGTTTA TCGCAACGAG CGCATCCACA AGGCCGTGAG CGTGAGCCGG
 ACCCGCAAAT AGCGTTGCTC GCGTAGGTGT TCCGGCACTC GCACTCGGCC
 12601 CGGCGCGAGC TCAGCGACCG CGAGCTGATG CACAGCCTGC AAAGGGCCCT
 GCCGCGCTCG AGTCGCTGGC GCTCGACTAC GTGTCGGACG TTTCCCGGGA
 12651 GGCTGGCACG GGCAGCGGCG ATAGAGAGGC CGAGTCCTAC TTTGACGCGG
 CCGACCGTGC CCGTCGCGCG TATCTCTCCG GCTCAGGATG AAAGTGCGCC
 12701 GCGCTGACCT GCGCTGGGCC CCAAGCCGAC GCGCCCTGGA GGCAGCTGGG
 CGCGACTGGA CGCGACCCGG GGTTCGGCTG CGCGGGACCT CCGTCGACCC
 12751 GCCGGACCTG GGCTGGCGGT GGCACCCGCG CGCGCTGGCA ACGTCGGCGG
 CGGCCTGGAC CCGACCGCCA CCGTGGGCGC GCGCGACCGT TGCAGCCGCC
 12801 CGTGGAGGAA TATGACGAGG ACGATGAGTA CGAGCCAGAG GACGGCGAGT
 GCACCTCCTT ATACTGCTCC TGCTACTCAT GCTCGGTCTC CTGCCGCTCA
 12851 ACTAAGCGGT GATGTTTCTG ATCAGATGAT GCAAGACGCA ACGGACCCGG
 TGATTGCGCA CTACAAAGAC TAGTCTACTA CGTTCTGCGT TGCCTGGGCC
 12901 CGGTGCGGGC GCGCTGCGAG AGCCAGCCGT CCGGCCTTAA CTCCACGGAC
 GCCACGCCCC CCGCGACGTC TCGGTGCGCA GGCCGGAATT GAGGTGCTCTG
 12951 GACTGGCGCC AGGTCATGGA CCGCATCATG TCGCTGACTG CGCGCAATCC
 CTGACCGCGG TCCAGTACCT GCGGTAGTAC AGCGACTGAC GCGCGTTAGG
 13001 TGACGCGTTC CGGCAGCAGC CGCAGGCCAA CCGGCTCTCC GCAATTCTGG
 ACTGCGCAAG GCCGTCGTCG GCGTCCGGTT GGCCGAGAGG CGTTAAGACC
 13051 AAGCGGTGGT CCCGGCGCGC GCAAACCCCA CGCACGAGAA GGTGCTGGCG
 TTCGCCACCA GGGCCGCGCG CGTTTGGGGT GCGTGCTCTT CCACGACCGC
 13101 ATCGTAAACG CGCTGGCCGA AAACAGGGCC ATCCGGCCCC ACGAGGCCGG
 TAGCATTTGC GCGACCGGCT TTTGTCCCGG TAGGCCGGGC TGCTCCGGCC
 13151 CCTGGTCTAC GACGCGCTGC TTCAGCGCGT GGCTCGTTAC AACAGCGGCA
 GGACCAGATG CTGCGCGACG AAGTCGCGCA CCGAGCAATG TTGTCGCCGT
 13201 ACGTGCAGAC CAACCTGGAC CGGCTGGTGG GGGATGTGCG CGAGGCCCGT
 TGCACGTCTG GTTGGACCTG GCCGACCACC CCCTACACGC GCTCCGGCAC

Figure 26 N

13301 ACTAAACGCC TTCCTGAGTA CACAGCCCCG CAACGTGCCG CGGGGACAGG
 TGATTTGCGG AAGGACTCAT GTGTCGGGCG GTTGCACGGC GCCCCTGTCC
 13351 AGGACTACAC CAACTTTGTG AGCGCACTGC GGCTAATGGT GACTGAGACA
 TCCTGATGTG GTTGAAACAC TCGCGTGACG CCGATTACCA CTGACTCTGT
 13401 CCGCAAAGTG AGGTGTACCA GTCTGGGCCA GACTATTTTT TCCAGACCAG
 GGCCTTTTAC TCCACATGGT CAGACCCGGT CTGATAAAAA AGGTC'TGGTC
 13451 TAGACAAGGC CTGCAGACCG TAAACCTGAG CCAGGCTTTC AAAA'ACTTGC
 ATCTGTTCCG GACGTCTGGC ATTTGGACTC GGTCCGAAAG TTTT'TGAACG
 13501 AGGGGCTGTG GGGGGTGCGG GCTCCACAG GCGACCGCGC GACCGTGTCT
 TCCCCGACAC CCCCCACGCC CGAGGGTGTC CGCTGGCGCG CTGGCACAGA
 13551 AGCTTGCTGA CGCCCAACTC GCGCCTGTTG CTGCTGCTAA TAGCGCCCTT
 TCGAACGACT GCGGGTTGAG CGCGGACAAC GACGACGATT ATCGCGGGAA
 13601 CACGGACAGT GGCAGCGTGT CCCGGGACAC ATACCTAGGT CACTTGCTGA
 GTGCCTGTCA CCGTCGCACA GGGCCCTGTG TATGGATCCA GTGAACGACT
 13651 CACTGTACCG CGAGGCCATA GGTCAGGCGC ATGTGGACGA GCATACTTTC
 GTGACATGGC GTC'CCGTAT CCAGTCCGCG TACACCTGCT CGTATGAAAG
 13701 CAGGAGATTA CAAGTGTCAG CCGCGCGCTG GGGCAGGAGG ACACGGGCAG
 GTCCTCTAAT GTTCACAGTC GGC'GCGGAC CCCGTCCTCC TGTGCCCGTC
 13751 CCTGGAGGCA ACCCTAAACT ACCTGCTGAC CAACCGGCGG CAGAAGATCC
 GGACCTCCGT TGGGATTTGA TGGACGACTG GTTGGCCGCC GTCTTCTAGG
 13801 CCTCGTTGCA CAGTTTAAAC AGCGAGGAGG AGCGCATTTT GCGCTACGTG
 GGAGCAACGT GTCAAATTTG TCGCTCCTCC TCGCGTAAAA CGCGATGCAC
 13851 CAGCAGAGCG TGAGCCTTAA CCTGATGCGC GACGGGGTAA CGCCCAGCGT
 GTCGTCTCGC ACTCGGAATT GGACTACGCG CTGCCCCATT GCGGGT'CGCA
 13901 GGCGCTGGAC ATGACCGCGC GCAACATGGA ACCGGGCATG TATGCCTCAA
 CCGCGACCTG TACTGGCGCG CGTTGTACCT TGGCCCGTAC ATACGGAGTT
 13951 ACCGGCCGTT TATCAACCGC CTAATGGACT ACTTG'CATCG CGCGGCCCGC
 TGGCCGGCAA ATAGTTGGCG GATTACCTGA TGAACGTAGC GCGCCGGCGG
 14001 GTGAACCCCG AGTATTTTAC CAATGCCATC TTGAACCCCG ACTGGCTACC
 CACTTGGGGC TCATAAAGTG GTTACGGTAG AACTTGGGCG TGACCGATGG
 14051 GCCCCCTGGT TTCTACACCG GGGGATTCGA GGTGCCCAGG GGTAACGATG
 CGGGGGACCA AAGATGTGGC CCCCTAAGCT CCACGGGCTC CCATTGCTAC
 14101 GATTCCTCTG GGACGACATA GACGACAGCG TGTTTTTCCC GCAACCGCAG
 CTAAGGAGAC CCTGCTGTAT CTGCTGTCGC ACAAAGGGG CGTTGGCGTC
 14151 ACCCTGCTAG AGTTGCAACA GCGCGAGCAG GCAGAGGCGG CGCTGCGAAA
 TGGGACGATC TCAACGTTGT CGCGCTCGTC CGTCTCCGCC GCGACGCTTT

Figure 260

| | | | | | |
|-------|-------------|------------|-------------|-------------|------------|
| 14251 | CGCGGTCAGA | TGCTAGTAGC | CCATTTCCAA | GCTTGATAGG | GTCTCTTACC |
| | GCGCCAGTCT | ACGATCATCG | GGTAAAGGTT | CGAACTATCC | CAGAGAATGG |
| 14301 | AGCACTCGCA | CCACCCGCCC | GCGCCTGCTG | GGCGAGGAGG | AGTACCTAAA |
| | TCGTGAGCGT | GGTGGGCGGG | CGCGGACGAC | CCGCTCCTCC | TCATGGATTT |
| 14351 | CAACTCGCTG | CTGCAGCCGC | AGCGCGAAAA | AAACCTGCCT | CCGGCATTTC |
| | GTTGAGCGAC | GACGTCGGCG | TCGCGCTTTT | TTTGGACGGA | GGCCGTAAAG |
| 14401 | CCAACAACGG | GATAGAGAGC | CTAGTGGACA | AGATGAGTAG | ATGGAAGACG |
| | GGTTGTTGCC | CTATCTCTCG | GATCACCTGT | TCTACTCATC | TACCTTCTGC |
| 14451 | TACGCGCAGG | AGCACAGGGA | CGTGCCAGGC | CCGCGCCCGC | CCACCCGTCG |
| | ATGCGCGTCC | TCGTGTCCCT | GCACGGTCCG | GGCGCGGGCG | GGTGGGCAGC |
| 14501 | TCAAAGGCAC | GACCGTCAGC | GGGGTCTGGT | GTGGGAGGAC | GATGACTCGG |
| | AGTTTCCGTG | CTGGCAGTCG | CCCCAGACCA | CACCCCTCCTG | CTACTGAGCC |
| 14551 | CAGACGACAG | CAGCGTCCTG | GATTTGGGAG | GGAGTGGCAA | CCCGTTTGCG |
| | GTCTGTCTGC | GTCGCAGGAC | CTAAACCCTC | CCTCACCGTT | GGGCAAACGC |
| 14601 | CACCTTCGCC | CCAGGCTGGG | GAGAATGTTT | TAAAAAAAAA | AAAAGCATGA |
| | GTGGAAGCGG | GGTCCGACCC | CTCTTACAAA | ATTTTTTTTT | TTTTCGTACT |
| 14651 | TGCAAAATAA | AAAACTCACC | AAGGCCATGG | CACCGAGCGT | TGGTTTTCTT |
| | ACGTTTTTATT | TTTTGAGTGG | TTCCGGTACC | GTGGCTCGCA | ACCAAAAGAA |
| 14701 | GTATTCCCCT | TAGTATGCGG | CGCGCGGCGA | TGTATGAGGA | AGGTCTCTCT |
| | CATAAGGGGA | ATCATACGCC | GCGCGCCGCT | ACATACTCCT | TCCAGGAGGA |
| 14751 | CCCTCCTACG | AGAGTGTGGT | GAGCGCGGCG | CCAGTGGCGG | CGGCGCTGGG |
| | GGGAGGATGC | TCTCACACCA | CTCGCGCCGC | GGTCACCGCC | GCCGCGACCC |
| 14801 | TTCTCCCTTC | GATGCTCCCC | TGGACCCGCC | GTTTGTGCCT | CCGCGGTACC |
| | AAGAGGGAAG | CTACGAGGGG | ACCTGGGCGG | CAAACACGGA | GGCGCCATGG |
| 14851 | TGCGGCCTAC | CGGGGGGAGA | AACAGCATCC | GTTACTCTGA | GTTGGCACCC |
| | ACGCCGGATG | GCCCCCTCT | TTGTCTGTAGG | CAATGAGACT | CAACCGTGGG |
| 14901 | CTATTTCGACA | CCACCCGTGT | GTACCTGGTG | GACAACAAGT | CAACGGATGT |
| | GATAAGCTGT | GGTGGGCACA | CATGGACCAC | CTGTTGTTCA | GTTGCCTACA |
| 14951 | GGCATCCCTG | AACTACCAGA | ACGACCACAG | CAACTTTCTG | ACCACGGTCA |
| | CCGTAGGGAC | TTGATGGTCT | TGCTGGTGTC | GTTGAAAGAC | TGGTGCCAGT |
| 15001 | TTCAAAACAA | TGACTACAGC | CCGGGGGAGG | CAAGCACACA | GACCATCAAT |
| | AAGTTTTTGT | ACTGATGTCT | GGCCCCCTCC | GTTCGTGTGT | CTGGTAGTTA |
| 15051 | CTTGACGACC | GGTCGCACTG | GGGCGGCGAC | CTGAAAACCA | TCCTGCATAC |
| | GAACGTCTGG | CCAGCGTGAC | CCCGCCGCTG | GACTTTTGGT | AGGACGTATG |
| 15101 | CAACATGCCA | AATGTGAACG | AGTTCATGTT | TACCAATAAG | TTTAAGGCGC |
| | GTTGTACGGT | TTACACTTGC | TCAAGTACAA | ATGGTTATTC | AAATTCCGCG |

Figure 26 P

15151 GGGTGATGGT GTCGCGCTTG CCTACTAAGG ACAATCAGGT GGAGCTGAAA
CCCCTACCA CAGCGCGAAC GGATGATTCC TGTTAGTCCA CCTCGACTTT

15201 TACGAGTGGG TGGAGTTCAC GCTGCCCCGAG GGCAACTACT CCGAGACCAT
ATGCTCACC ACCTCAAGTG CGACGGGCTC CCGTTGATGA GGCTCTGGTA

15251 GACCATAGAC CTTATGAACA ACGCGATCGT GGAGCACTAC TTGAAAGTGG
CTGGTATCTG GAATACTTGT TGCCTAGCA CCTCGTGATG AACTTTCACC

15301 GCAGACAGAA CGGGGTTCCTG GAAAGCGACA TCGGGGTAAA GTTTGACACC
CGTCTGTCTT GCCCCAAGAC CTTTCGCTGT AGCCCCATTT CAAACTGTGG

15351 CGCAACTTCA GACTGGGGTT TGACCCCGTC ACTGGTCTTG TCATGCCTGG
GCGTTGAAGT CTGACCCCAA ACTGGGGCAG TGACCAGAAC AGTACGGACC

15401 GGTATATACA AACGAAGCCT TCCATCCAGA CATCATTTTG CTGCCAGGAT
CCATATATGT TTGCTTCGGA AGGTAGGTCT GTAGTAAAC GACGGTCCTA

15451 GCGGGGTGGA CTTACCCAC AGCCGCCTGA GCAACTTGTT GGGCATCCGC
CGCCCCACCT GAAGTGGGTG TCGGCGGACT CGTTGAACAA CCCGTAGGCG

15501 AAGCGGCAAC CTTCCAGGA GGGCTTTAGG ATCACCTACG ATGATCTGGA
TTGCCCGTTG GGAAGGTCCT CCCGAAATCC TAGTGGATGC TACTAGACCT

15551 GGGTGGTAAC ATTCCCGCAC TGTTGGATGT GGACGCCTAC CAGGCGAGCT
CCCACCATTG TAAGGGCGTG ACAACCTACA CCTGCGGATG GTCCGCTCGA

15601 TGAAAGATGA CACCGAACAG GCGGGGGTG GCGCAGGCGG CAGCAACAGC
ACTTCTACT GTGGCTTGTC CCGCCCCAC CGCGTCCGCC GTCGTTGTCG

15651 AGTGGCAGCG GCGCGGAAGA GAACTCCAAC GCGGCAGCCG CGGCAATGCA
TCACCGTCGC CGCGCCTTCT CTTGAGGTTG CGCCGTCGGC GCCGTTACGT

15701 GCCGGTGGAG GACATGAACG ATCATGCCAT TCGCGGCGAC ACCTTTGCCA
CGGCCACCTC CTGTACTTGC TAGTACGGTA AGCGCCGCTG TGGAAACGGT

15751 CACGGGCTGA GGAGAAGCGC GCTGAGGCCG AAGCAGCGGC CGAAGCTGCC
GTGCCCGACT CCTCTTCGCG CGACTCCGGC TTCGTCGCCG GCTTCGACGG

15801 GCCCCCGCTG CGCAACCCGA GGTCGAGAAG CCTCAGAAGA AACC GG TGAT
CGGGGGCGAC GCGTTGGGCT CCAGCTCTTC GGAGTCTTCT TTGGCCACTA

15851 CAAACCCCTG ACAGAGGACA GCAAGAAACG CAGTTACAAC CTAATAAGCA
GTTTGGGGAC TGTCTCCTGT CGTTCTTTGC GTCAATGTTG GATTATTCTG

15901 ATGACAGCAC CTTACCCAG TACCGCAGCT GGTACCTTGC ATACAAC TAC
TACTGTCTG GAAGTGGGTC ATGGCGTCGA CCATGGAACG TATGTTGATG

15951 GGCGACCCCTC AGACCGGAAT CCGCTCATGG ACCCTGCTTT GCACTCCTGA
CCGCTGGGAG TCTGGCCTTA GGCGAGTACC TGGGACGAAA CGTGAGGACT

16001 CGTAACCTGC GGCTCGGAGC AGGTCTACTG GTCGTTGCCA GACATGATGC
GCATTGGACG CCGAGCCTCG TCCAGATGAC CAGCAACGGT CTGTACTACG

16051 AAGACCCCGT GACCTTCCGC TCCACGCGCC AGATCAGCAA CTTTCCGGTG
TTCTGGGGCA CTGGAAGGCG AGGTGCGCGG TCTAGTCGTT GAAAGGCCAC

Figure 26 Q

| | | | | | |
|-------|------------|-------------|------------|------------|------------|
| 16151 | GGCCGTCTAC | TCCCAACTCA | TCCGCCAGTT | TACCTCTCTG | ACCCACGTGT |
| | CCGGCAGATG | AGGGTTGAGT | AGGCGGTCAA | ATGGAGAGAC | TGGGTGCACA |
| 16201 | TCAATCGCTT | TCCCGAGAAC | CAGATTTTGG | CGCGCCCGCC | AGCCCCCACC |
| | AGTTAGCGAA | AGGGCTCTTG | GTCTAAAACC | GCGCGGGCGG | TCGGGGGTGG |
| 16251 | ATCACCACCG | TCAGTGAAAA | CGTTCTTGCT | CTCACAGATC | ACGGGACGCT |
| | TAGTGGTGGC | AGTCACTTTT | GCAAGGACGA | GAGTGTCTAG | TGCCCTGCGA |
| 16301 | ACCCTGCGC | AACAGCATCG | GAGGAGTCCA | GCGAGTGACC | ATTACTGACG |
| | TGGCGACCG | TTGTCTAGC | CTCCTCAGGT | CGCTCACTGG | TAATGACTGC |
| 16351 | CCAGACGCCG | CACCTGCCCC | TACGTTTACA | AGGCCCTGGG | CATAGTCTCG |
| | GGTCTGCGGC | GTGGACGGGG | ATGCAAATGT | TCCGGGACCC | GTATCAGAGC |
| 16401 | CCGCGCGTCC | TATCGAGCCG | CACTTTTTGA | GCAAGCATGT | CCATCCTTAT |
| | GGCGCGCAGG | ATAGCTCGGC | GTGAAAAACT | CGTTCGTACA | GGTAGGAATA |
| 16451 | ATCGCCCAGC | AATAACACAG | GCTGGGGCCT | GCGCTTCCCA | AGCAAGATGT |
| | TAGCGGGTCG | TTATTGTGTC | CGACCCCGGA | CGCGAAGGGT | TCGTTCTACA |
| 16501 | TTGGCGGGGC | CAAGAAGCGC | TCCGACCAAC | ACCCAGTGCG | CGTGCGCGGG |
| | AACCGCCCCG | GTTCTTCGCG | AGGCTGGTTG | TGGGTCACGC | GCACGCGCCC |
| 16551 | CACTACCGCG | CGCCCTGGGG | CGCGCACAAA | CGCGGCCGCA | CTGGGCGCAC |
| | GTGATGGCGC | GCGGGACCCC | GCGCGTGTTT | GCGCCGGCGT | GACCCGCGTG |
| 16601 | CACCGTCGAT | GACGCCATCG | ACGCGGTGGT | GGAGGAGGCG | CGCAACTACA |
| | GTGGCAGCTA | CTGCGGTAGC | TGCGCCACCA | CCTCCTCCGC | GCGTTGATGT |
| 16651 | CGCCACGCC | GCCACCAGTG | TCCACAGTGG | ACGCGGCCAT | TCAGACCGTG |
| | GCGGGTGCGG | CGGTGGTCAC | AGGTGTCACC | TGCGCCGGTA | AGTCTGGCAC |
| 16701 | GTGCGCGGAG | CCCGGCGCTA | TGCTAAAATG | AAGAGACGGC | GGAGGCGCGT |
| | CACGCGCCTC | GGGCGCGAT | ACGATTTTAC | TTCTCTGCCG | CCTCCGCGCA |
| 16751 | AGCACGTCGC | CACCGCCGCC | GACCCGGCAC | TGCCGCCCAA | CGCGCGGCGG |
| | TCGTGCAGCG | GTGGCGGCGG | CTGGGCGGTG | ACGGCGGGTT | GCGCGCCGCC |
| 16801 | CGGCCCTGCT | TAACCGCGCA | CGTCGCACCG | GCCGACGGGC | GGCCATGCGG |
| | GCCGGGACGA | ATTGGCGCGT | GCAGCGTGGC | CGGCTGCCCC | CCGGTACGCC |
| 16851 | GCCGCTCGAA | GGCTGGCCGC | GGGTATTGTC | ACTGTGCCCC | CCAGGTCCAG |
| | CGGCGAGCTT | CCGACCGGCG | CCCATAACAG | TGACACGGGG | GGTCCAGGTC |
| 16901 | GCGACGAGCG | GCCGCCCGCAG | CAGCCGCGGC | CATTAGTGCT | ATGACTCAGG |
| | CGCTGCTCGC | CGGCGGCGTC | GTCGGCGCCG | GTAATCACGA | TACTGAGTCC |
| 16951 | GTCGCAGGGG | CAACGTGTAT | TGGGTGCGCG | ACTCGGTTAG | CGGCCTGCGC |
| | CAGCGTCCCC | GTTGCACATA | ACCCACGCGC | TGAGCCAATC | GCCGGACGCG |
| 17001 | GTGCCCCTGC | GCACCCGCCC | CCCGCGCAAC | TAGATTGCAA | GAAAAAACTA |
| | CACGGGCACG | CGTGGGCGGG | GGGCGCGTTG | ATCTAACGTT | CTTTTTTGAT |

Figure 26 R

| | | | | | |
|-------|-------------|------------|-------------|-------------|-------------|
| 17101 | CTATGTCCAA | GCGCAAAATC | AAAGAAGAGA | TGCTCCAGGT | CATCGCGCCG |
| | GATACAGGTT | CGCGTTTTAG | TTTCTTCTCT | ACGAGGTCCA | GTAGCGCGGC |
| 17151 | GAGATCTATG | GGCCCCCGAA | GAAGGAAGAG | CAGGATTACA | AGCCCCGAAA |
| | CTCTAGATAC | CGGGGGGCTT | CTTCCTTCTC | GTCCCTAATGT | TCGGGGCTTT |
| 17201 | GCTAAAGCGG | GTCAAAAAGA | AAAAGAAAGA | TGATGATGAT | GAACTTGACG |
| | CGATTTTCGCC | CAGTTTTTCT | TTTTCTTTCT | ACTACTACTA | CTTGAACTGC |
| 17251 | ACGAGGTGGA | ACTGCTGCAC | GCTACCGCGC | CCAGGCGACG | GGTACAGTGG |
| | TGCTCCACCT | TGACGACGTG | CGATGGCGCG | GGTCCGCTGC | CCATGTCACC |
| 17301 | AAAGGTCGAC | GCGTAAAACG | TGTTTTGCGA | CCCGGCACCA | CCGTAGTCTT |
| | TTTCCAGCTG | CGCATTTTGC | ACAAAACGCT | GGGCCGTGGT | GGCATCAGAA |
| 17351 | TACGCCCCGT | GAGCGCTCCA | CCCGCACCTA | CAAGCGCGTG | TATGATGAGG |
| | ATGCGGGCCA | CTCGCGAGGT | GGCGGTGGAT | GTTGCGGCAC | ATACTACTCC |
| 17401 | TGTACGGCGA | CGAGGACCTG | CTTGAGCAGG | CCAACGAGCG | CCTCGGGGAG |
| | ACATGCCGCT | GCTCCTGGAC | GAACTCGTCC | GGTTGCTCGC | GGAGCCCCCTC |
| 17451 | TTTGCTTACG | GAAAGCGGCA | TAAGGACATG | CTGGCGTTGC | CGCTGGACGA |
| | AAACGGATGC | CTTTCGCCGT | ATTCTGTAC | GACCGCAACG | GCGACCTGCT |
| 17501 | GGGCAACCCA | ACACCTAGCC | TAAAGCCCGT | AACACTGCAG | CAGGTGCTGC |
| | CCCGTTGGGT | TGTGGATCGG | ATTTTCGGGCA | TTGTGACGTC | GTCCACGACG |
| 17551 | CCGCGCTTGC | ACCGTCCGAA | GAAAAGCGCG | GCCTAAAGCG | CGAGTCTGGT |
| | GGCGCGAACG | TGGCAGGCTT | CTTTTCGCGC | CGGATTTGCG | GCTCAGACCA |
| 17601 | GACTTGGCAC | CCACCGTGCA | GCTGATGGTA | CCCAAGCGCC | AGCGACTGGA |
| | CTGAACCGTG | GGTGGCACGT | CGACTACCAT | GGGTTGCGCG | TCGCTGACCT |
| 17651 | AGATGTCCTG | GAAAAAATGA | CCGTGGAACC | TGGGCTGGAG | CCCGAGGTCC |
| | TCTACAGAAC | CTTTTTTACT | GGCACCTTGG | ACCCGACCTC | GGGCTCCAGG |
| 17701 | GCGTGCGGCC | AATCAAGCAG | GTGGCGCCGG | GACTGGGCGT | GCAGACCGTG |
| | CGCACGCCGG | TTAGTTCGTC | CACCGCGGCC | CTGACCCGCA | CGTCTGGCAC |
| 17751 | GACGTTTACA | TACCCACTAC | CAGTAGCACC | AGTATTGCCA | CCGCCACAGA |
| | CTGCAAGTCT | ATGGGTGATG | GTCATCGTGG | TCATAACGGT | GGCGGTGTCT |
| 17801 | GGGCATGGAG | ACACAAACGT | CCCCGGTTGC | CTCAGCGGTG | GCGGATGCCG |
| | CCCGTACCTC | TGTGTTTGCA | GGGGCCAACG | GAGTCGCCAC | CGCCTACGGC |
| 17851 | CGGTGCAGGC | GGTCGCTGCG | GCCGCGTCCA | AGACCTCTAC | GGAGGTGCAA |
| | GCCACGTCCG | CCAGCGACGC | CGGCGCAGGT | TCTGGAGATG | CCTCCACGTT |
| 17901 | ACGGACCCGT | GGATGTTTCG | CGTTTCAGCC | CCCCGGCGCC | CGCGCCGTTC |
| | TGCCCTGGGCA | CCTACAAAGC | GCAAAGTCGG | GGGGCCGCGG | GCGCGGCAAG |
| 17951 | GAGGAAGTAC | GGCGCCGCCA | GCGCGCTACT | GCCCCGAATAT | GCCCTACATC |
| | CTCCTTCATG | CCGCGGCGGT | GCGCGCATGA | CGGGCTTATA | CGGGATGTAG |

Figure 26S

18051 AGACGAGCAA CTACCCGACG CCGAACCACC ACTGGAACCC GCCGCCGCCG
 TCTGCTCGTT GATGGGCTGC GGCTTGGTGG TGACCTTGGG CGGCGGCGGC

18101 TCGCCGTCGC CAGCCCGTGC TGGCCCCGAT TTCCGTGCGC AGGGTGGCTC
 AGCGGCAGCG GTCGGGCACG ACCGGGGCTA AAGGCACGCG TCCCACCGAG

18151 GCGAAGGAGG CAGGACCCCTG GTGCTGCCAA CAGCGCGCTA CCACCCACAGC
 CGCTTCCTCC GTCCTGGGAC CACGACGGTT GTCGCGCGAT GGTGGGGTGC

18201 ATCGTTTAAA AGCCGGTCTT TGTGGTTCTT GCAGATATGG CCCTCACCTG
 TAGCAAATTT TCGGCCAGAA ACACCAAGAA CGTCTATACC GGGAGTGGAC

18251 CCGCCTCCGT TTCCCGGTGC CGGGATTCCG AGGAAGAATG CACCGTAGGA
 GGCGGAGGCA AAGGGCCACG GCCCTAAGGC TCCTTCTTAC GTGGCATCCT

18301 GGGGCATGGC CGGCCACGGC CTGACGGGCG GCATGCGTCG TGCGCACCAC
 CCCCCTACCG GCCGGTGCCG GACTGCCCCG CGTACGCAGC ACGCGTGGTG

18351 CGGCGGCGGC GCGCGTCGCA CCGTCGCATG CGCGGCGGTA TCCTGCCCCCT
 GCCGCCGCCG CGCGCAGCGT GGCAGCGTAC GCGCCGCCAT AGGACGGGGA

18401 CCTTATTCCA CTGATCGCCG CGGCGATTGG CGCCGTGCCC GGAATTGCAT
 GGAATAAGGT GACTAGCGGC GCCGCTAACC GCGGCACGGG CCTTAACGTA

18451 CCGTGGCCTT GCAGGCGCAG AGACACTGAT TAAAAACAAG TTGCATGTGG
 GGCACCGGAA CGTCCGCGTC TCTGTGACTA ATTTTGTTC AACGTACACC

18501 AAAAAATCAA ATAAAAAGTC TGGACTCTCA CGCTCGCTTG GTCCTGTAAC
 TTTTATAGTT TATTTTTCAG ACCTGAGAGT GCGAGCGAAC CAGGACATTG

18551 TATTTGTAG AATGGAAGAC ATCAACTTTG CGTCTCTGGC CCCGCGACAC
 ATAAAACATC TTACCTTCTG TAGTTGAAAC GCAGAGACCG GGGCGCTGTG

18601 GGCTCGCGCC CGTTCATGGG AAACCTGGCA GATATCGGCA CCAGCAATAT
 CCGAGCGCGG GCAAGTACCC TTTGACCGTT CTATAGCCGT GGTCGTTATA

18651 GAGCGGTGGC GCCTTCAGCT GGGGCTCGCT GTGGAGCGGC ATTAAAAATT
 CTCGCCACCG CGGAAGTCGA CCCCAGCGCA CACCTCGCCG TAATTTTAA

18701 TCGGTTCCAC CGTTAAGAAC TATGGCAGCA AGGCCTGGAA CAGCAGCACA
 AGCCAAGGTG GCAATTCTTG ATACCGTCGT TCCGGACCTT GTCGTCGTGT

18751 GGCCAGATGC TGAGGGATAA GTTGAAAGAG CAAAATTTCC AACAAAAGGT
 CCGGTCTACG ACTCCCTATT CAACTTCTC GTTTTAAAGG TTGTTTCCA

18801 GGTAGATGGC CTGGCCTCTG GCATTAGCGG GGTGGTGGAC CTGGCCAACC
 CCATCTACCG GACCGGAGAC CGTAATCGCC CCACCACCTG GACCGGTGCG

18851 AGGCAGTGCA AAATAAGATT AACAGTAAGC TTGATCCCCG CCCTCCCGTA
 TCCGTCACGT TTTATTCTAA TTGTCATTCG AACTAGGGGC GGGAGGGCAT

18901 GAGGAGCCTC CACCGGCCGT GGAGACAGTG TCTCCAGAGG GCGGTGGCGA
 CTCCTCGGAG GTGGCCGGCA CCTCTGTCAC AGAGGTCTCC CCGCACCGCT

Figure 26.T

19001 AGCCTCCCTC GTACGAGGAG GCACTAAAGC AAGGCCTGCC CACCACCCGT
TCGGAGGGAG CATGCTCCTC CGTGATTTCG TTCCGGACGG GTGGTGGGCA

19051 CCCATCGCGC CCATGGCTAC CGGAGTGCTG GGCCAGCACA CACCCGTAAC
GGGTAGCGCG GGTACCGATG GCCTCACGAC CCGGTCGTGT GTGGGCATTG

19101 GCTGGACCTG CCTCCCCCG CCGACACCCA GCAGAAACCT GTGCTGCCAG
CGACCTGGAC GGAGGGGGGC GGCTGTGGGT CGTCTTTGGA CACGACGGTC

19151 GCCCGACCGC CGTTGTTGTA ACCCGTCCTA GCCGCGCGTC CCTGCGCCGC
CGGGCTGGCG GCAACAACAT TGGGCAGGAT CGGCGCGCAG GGACGCGGCG

19201 GCCGCCAGCG GTCCGCGATC GTTGCGGGCC GTAGCCAGTG GCAACTGGCA
CGGCGGTGCG CAGGCGCTAG CAACGCCGGG CATCGGTCAC CGTTGACCGT

19251 AAGCACACTG AACAGCATCG TGGGTCTGGG GGTGCAATCC CTGAAGCGCC
TTCGTGTGAC TTGTCTGATG ACCCAGACCC CCACGTTAGG GACTTCGCGG

19301 GACGATGCTT CTGATAGCTA ACGTGTGCTA TGTGTGTCAT GTATGCGTCC
CTGCTACGAA GACTATCGAT TGCACAGCAT ACACACAGTA CATACGCAGG

19351 ATGTCGCCGC CAGAGGAGCT GCTGAGCCGC CGCGCGCCCG CTTTCCAAGA
TACAGCGGCG GTCTCCTCGA CGACTCGGCG GCGCGCGGGC GAAAGGTTCT

19401 TGGCTACCCC TTCGATGATG CCGCAGTGGT CTTACATGCA CATCTCGGGC
ACCGATGGGG AAGCTACTAC GGCCTCACCA GAATGTACGT GTAGAGCCCG

19451 CAGGACGCCT CGGAGTACCT GAGCCCCGGG CTGGTGCACT TTGCCCGCGC
GTCCTGCGGA GCCTCATGGA CTCGGGGCCC GACCACGTCA AACGGGCGCG

19501 CACCGAGACG TACTTCAGCC TGAATAACAA GTTTAGAAAC CCCACGGTGG
GTGGCTCTGC ATGAAGTCGG ACTTATTGTT CAAATCTTTG GGGTGCCACC

19551 CGCCTACGCA CGACGTGACC ACAGACCGGT CCCAGCGTTT GACGCTGCGG
GCGGATGCGT GCTGCACTGG TGTCTGGCCA GGGTCGCAA CTGCGACGCC

19601 TTCATCCCTG TGGACCGTGA GGATACTGCG TACTCGTACA AGGCGCGGTT
AAGTAGGGAC ACCTGGCACT CCTATGACGC ATGAGCATGT TCCGCGCCAA

19651 CACCCTAGCT GTGGGTGATA ACCGTGTGCT GGACATGGCT TCCACGTACT
GTGGGATCGA CACCCACTAT TGGCACACGA CCTGTACCGA AGGTGCATGA

19701 TTGACATCCG CGGCGTGCTG GACAGGGGCC CTACTTTTAA GCCCTACTCT
AACTGTAGGC GCCGCACGAC CTGTCCCCGG GATGAAAATT CGGGATGAGA

19751 GGCCTGCGCT ACAACGCCCT GGCTCCCAAG GGTGCCCCAA ATCCTTGCGA
CCGTGACGGA TGTTGCGGGA CCGAGGGTTC CCACGGGGTT TAGGAACGCT

19801 ATGGGATGAA GCTGCTACTG CTCTTGAAAT AAACCTAGAA GAAGAGGACG
TACCCTACTT CGACGATGAC GAGAACTTTA TTTGGATCTT CTTCTCCTGC

19851 ATGACAACGA AGACGAAGTA GACGAGCAAG CTGAGCAGCA AAAAATCACC
TACTGTTGCT TCTGCTTCAT CTGCTCGTTC GACTCGTCGT TTTTGTAGTG

Figure 26 u

19951 TCAAATAGGT GTCGAAGGTC AAACACCTAA ATATGCCGAT AAAACATTTT
AGTTTATCCA CAGCTTCCAG TTTGTGGATT TATACGGCTA TTTTGTAAAG

20001 AACCTGAACC TCAAATAGGA GAATCTCAGT GGTACGAAAC AGAAATTAAT
TTGGACTTGG AGTTTATCCT CTTAGAGTCA CCATGCTTTG TCTTTAATTA

20051 CATGCAGCTG GGAGAGTCCT AAAAAAGACT ACCCCAATGA AACCATGTTA
GTACGTCGAC CCTCTCAGGA TTTTTTCTGA TGGGGTTACT TTGGTACAAT

20101 CGGTTCATAT GCAAAACCCA CAAATGAAAA TGGAGGGCAA GGCATTCTTG
GCCAAGTATA CGTTTTGGGT GTTTACTTTT ACCTCCCGTT CCGTAA-GAAC

20151 TAAAGCAACA AAATGGAAAG CTAGAAAGTC AAGTGGAAAT GCAATTTTTTC
ATTTCGTTGT TTTACCTTTC GATCTTTCAG TTCACCTTTA CGTTAAAAAG

20201 TCAACTACTG AGGCAGCCGC AGGCAATGGT GATAACTTGA CTCCTAAAGT
AGTTGATGAC TCCGTCGGCG TCCGTTACCA CTATTGAACT GAGGATTTCA

20251 GGTATTGTAC AGTGAAGATG TAGATATAGA AACCCAGAC ACTCATATTT
CCATAACATG TCACTTCTAC ATCTATATCT TTGGGGTCTG TGAGTATAAA

20301 CTTACATGCC CACTATTAAG GAAGGTAAC CACGAGAACT AATGGGCCAA
GAATGTACGG GTGATAATTC CTTCCATTGA GTGCTCTTGA TTACCCGGTT

20351 CAATCTATGC CCAACAGGCC TAATTACATT GCTTTTAGGG ACAATTTTAT
GTTAGATACG GGTGTGCCG ATTAATGTAA CGAAAATCCC TGTAAAAATA

20401 TGGTCTAATG TATTACAACA GCACGGGTAA TATGGGTGTT CTGGCGGGCC
ACCAGATTAC ATAATGTTGT CGTGCCCAT TATACCCACAA GACCGCCCGG

20451 AAGCATCGCA GTTGAATGCT GTTGTAGATT TGCAAGACAG AAACACAGAG
TTCGTAGCGT CAACTTACGA CAACATCTAA ACGTTCTGTC TTTGTGTCTC

20501 CTTTCATACC AGCTTTTGCT TGATTCCATT GGTGATAGAA CCAGGTACTT
GAAAGTATGG TCGAAAACGA ACTAAGGTAA CCACTATCTT GGTCCATGAA

20551 TTCTATGTGG AATCAGGCTG TTGACAGCTA TGATCCAGAT GTTAGAATTA
AAGATACACC TTAGTCCGAC AACTGTGCGAT ACTAGGTCTA CAATCTTAAT

20601 TTGAAAATCA TGGAACGTAA GATGAACTTC CAAATTACTG CTTTCCACTG
AACTTTTAGT ACCTTGACTT CTACTTGAAG GTTTAATGAC GAAAGGTGAC

20651 GGAGGTGTGA TTAATACAGA GACTCTTACC AAGGTAAAAC CTAAAACAGG
CCTCCACACT AATTATGTCT CTGAGAATGG TTCCATTTTG GATTTTGTCC

20701 TCAGGAAAAT GGATGGGAAA AAGATGCTAC AGAATTTTCA GATAAAAATG
AGTCCTTTTA CCTACCTTTT TTCTACGATG TCTTAAAAGT CTATTTTTAC

20751 AAATAAGAGT TGGAAATAAT TTTGCCATGG AAATCAATCT AAATGCCAAC
TTTATTCTCA ACCTTTATTA AAACGGTACC TTTAGTTAGA TTTACGGTTG

20801 CTGTGGAGAA ATTTCTGTGA CTCCAACATA GCGCTGTATT TGCCCGACAA
GACACCTCTT TAAAGGACAT GAGGTTGTAT CGCGACATAA ACGGGCTGTT

Figure 26 v

20901 ACGACTACAT GAACAAGCGA GTGGTGGCTC CCGGGCTAGT GGACTGCTAC
 TGCTGATGTA CTTGTTTCGCT CACCACCGAG GGCCCCGATCA CCTGACGATG

20951 ATTAACCTTG GAGCACGCTG GTCCCTTGAC TATATGGACA ACGTCAACCC
 TAATTGGAAC CTCGTGCGAC CAGGGAAC TG ATATACCTGT TGCAGTTGGG

21001 ATTTAACCAC CACCGCAATG CTGGCCTGCG CTACCGCTCA ATGTTGCTGG
 TAAATTGGTG GTGGCGTTAC GACCGGACGC GATGGCGAGT TACAACGACC

21051 GCAATGGTCG CTATGTGCCC TTCCACATCC AGGTGCCTCA GAAGTTCTTT
 CGTTACCAGC GATACACGGG AAGGTGTAGG TCCACGGAGT CTTCAAGAAA

21101 GCCATTAAAA ACCTCCTTCT CCTGCCGGGC TCATACACCT ACGAGTGGAA
 CGGTAATTTT TGGAGGAAGA GGACGGCCCG AGTATGTGGA TGCTCACCTT

21151 CTTCAGGAAG GATGTTAACA TGGTTCTGCA GAGCTCCCTA GGAAATGACC
 GAAGTCCTTC CTACAATTGT ACCAAGACGT CTCGAGGGAT CCTTTACTGG

21201 TAAGGGTTGA CGGAGCCAGC ATTAAGTTTG ATAGCATTTG CCTTTACGCC
 ATTCCCAACT GCCTCGGTG TAATTCAAAC TATCGTAAAC GGAAATGCGG

21251 ACCTTCTTCC CCATGGCCCA CAACACCGCC TCCACGCTTG AGGCCATGCT
 TGGAGAAGG GGTACCGGGT GTTGTGGCGG AGGTGCGAAC TCCGGTACGA

21301 TAGAAACGAC ACCAACGACC AGTCCTTTAA CGACTATCTC TCCGCCGCCA
 ATCTTTGCTG TGGTTGCTGG TCAGGAAATT GCTGATAGAG AGGCGGCGGT

21351 ACATGCTCTA CCCTATACCC GCCAACGCTA CCAACGTGCC CATATCCATC
 TGTACGAGAT GGGATATGGG CGGTTGCGAT GGTGACACGG GTATAGGTAG

21401 CCCTCCCGCA ACTGGGCGGC TTTCCGCGGC TGGGCCTTCA CGCGCCTTAA
 GGGAGGGCGT TGACCCGCCG AAAGGCGCCG ACCCGGAAGT GCGCGGAATT

21451 GACTAAGGAA ACCCATCAC TGGGCTCGGG CTACGACCCT TATTACACCT
 CTGATTCCTT TGGGGTAGTG ACCCGAGCCC GATGCTGGGA ATAATGTGGA

21501 ACTCTGGCTC TATACCCTAC CTAGATGGAA CCTTTTACCT CAACCACACC
 TGAGACCGAG ATATGGGATG GATCTACCTT GGAAATGGA GTTGGTGTGG

21551 TTTAAGAAGG TGGCCATTAC CTTTGA CTCT TCTGTGCTAGCT GGCCTGGCAA
 AAATTCTTCC ACCGTAATG GAAACTGAGA AGACAGTCGA CCGGACCGTT

21601 TGACCGCCTG CTTACCCCCA ACGAGTTTGA AATTAAGCGC TCAGTTGACG
 ACTGGCGGAC GAATGGGGGT TGCTCAAAC TTAATTCGCG AGTCAACTGC

21651 GGGAGGGTTA CAACGTTGCC CAGTGTAACA TGACCAAAGA CTGGTTCTTG
 CCCTCCCAAT GTTGCAACGG GTCACATTGT ACTGGTTTCT GACCAAGGAC

21701 GTACAAATGC TAGCTAACTA TAACATTGGC TACCAGGGCT TCTATATCCC
 CATGTTTACG ATCGATTGAT ATTGTAACCG ATGGTCCCGA AGATATAGGG

21751 AGAGAGCTAC AAGGACCGCA TGTACTCCTT CTTTAGAAAC TTCCAGCCCA
 TCTCTCGATG TTCCTGGCGT ACATGAGGAA GAAATCTTTG AAGGTCGGGT

Figure 26 W

| | | | | | |
|-------|-------------|-------------|------------|------------|------------|
| 21851 | GGCATCCTAC | ACCAACACAA | CAACTCTGGA | TTTGTGGCT | ACCTTGCCCC |
| | CCGTAGGATG | TGGTTGTGTT | GTTGAGACCT | AAACAACCGA | TGGAACGGGG |
| 21901 | CACCATGCGC | GAAGGACAGG | CCTACCCTGC | TAACTTCCCC | TATCCGCTTA |
| | GTGGTACGCG | CTTCCTGTCC | GGATGGGACG | ATTGAAGGGG | ATAGGCGAAT |
| 21951 | TAGGCAAGAC | CGCAGTTGAC | AGCATTACCC | AGAAAAAGTT | TCTTTGCGAT |
| | ATCCGTTCTG | GCGTCAACTG | TCGTAATGGG | TCTTTTCAA | AGAAACGCTA |
| 22001 | CGCACCTTTT | GGCGCATCCC | ATTCTCCAGT | AACTTTATGT | CCATGGGCGC |
| | GCGTGGGAAA | CCGCGTAGGG | TAAGAGGTCA | TTGAAATACA | GGTACCCGCG |
| 22051 | ACTCACAGAC | CTGGGCCAAA | ACCTTCTCTA | CGCCAACTCC | GCCCACGCGC |
| | TGAGTGTCTG | GACCCGGTTT | TGGAAGAGAT | GCGGTTGAGG | CGGGTGCGCG |
| 22101 | TAGACATGAC | TTTTGAGGTG | GATCCCATGG | ACGAGCCCAC | CCTTCTTTAT |
| | ATCTGTACTG | AAAACCTCCAC | CTAGGTACC | TGCTCGGGTG | GGAAGAAATA |
| 22151 | GTTTTGTTTG | AAGTCTTTGA | CGTGGTCCGT | GTGCACCAGC | CGCACCGCGG |
| | CAAAACAAAC | TTCAGAACT | GCACCAGGCA | CACGTGGTCG | GCGTGGCGCC |
| 22201 | CGTCATCGAA | ACCGTGTACC | TGCGCACGCC | CTTCTCGGCC | GGCAACGCCA |
| | GCAGTAGCTT | TGGCACATGG | ACGCGTGCGG | GAAGAGCCGG | CCGTTGCGGT |
| 22251 | CAACATAAAG | AAGCAAGCAA | CATCAACAAC | AGCTGCCGCC | ATGGGCTCCA |
| | GTTGTATTTC | TTCGTTTCGT | GTAGTTGTTG | TCGACGGCGG | TACCCGAGGT |
| 22301 | GTGAGCAGGA | ACTGAAAGCC | ATTGTCAAAG | ATCTTGGTTG | TGGGCCATAT |
| | CACCTCGTCCT | TGACTTTCGG | TAACAGTTTC | TAGAACCAAC | ACCCGGTATA |
| 22351 | TTTTTGGGCA | CCTATGACAA | GCGCTTTCCA | GGCTTTGTTT | CTCCACACAA |
| | AAAAACCCGT | GGATACTGTT | CGCGAAAGGT | CCGAAACAAA | GAGGTGTGTT |
| 22401 | GCTCGCCTGC | GCCATAGTCA | ATACGGCCGG | TCGCGAGACT | GGGGGCGTAC |
| | CGAGCGGACG | CGGTATCAGT | TATGCCGGCC | AGCGCTCTGA | CCCCGCGATG |
| 22451 | ACTGGATGGC | CTTTGCCTGG | AACCCGCACT | CAAAAACATG | CTACCTCTTT |
| | TGACCTACCG | GAAACGGACC | TTGGGCGTGA | GTTTTTGTAC | GATGGAGAAA |
| 22501 | GAGCCCTTTG | GCTTTTCTGA | CCAGCGACTC | AAGCAGGTTT | ACCACTTTGA |
| | CTCGGGAAAC | CGAAAAGACT | GGTCGCTGAG | TTCGTCCAAA | TGGTCAAAC |
| 22551 | GTACGAGTCA | CTCCTGCGCC | GTAGCGCCAT | TGCTTCTTCC | CCCGACCGCT |
| | CATGCTCAGT | GAGGACGCGG | CATCGCGGTA | ACGAAGAAGG | GGGCTGGCGA |
| 22601 | GTATAACGCT | GGAAAAGTCC | ACCCAAAGCG | TACAGGGGCC | CAACTCGGCC |
| | CATATTGCGA | CCTTTTCAGG | TGGGTTTCGC | ATGTCCCCGG | GTTGAGCCGG |
| 22651 | GCCTGTGGAC | TATTCTGCTG | CATGTTTCTC | CACGCCTTTG | CCAACCTGGC |
| | CGGACACCTG | ATAAGACGAC | GTACAAAGAG | GTGCGGAAAC | GGTTGACCGG |
| 22701 | CCAAACTCCC | ATGGATCACA | ACCCACCAT | GAACCTTATT | ACCGGGGTAC |
| | GTTTTGAGGG | TACCTAGTGT | TGGGGTGGTA | CTTGAATAA | TGGCCCCATG |

Figure 26 X

22801 CAGGAACAGC TCTACAGCTT CCTGGAGCGC CACTCGCCCT ACTTCGCGAG
GTCTTGTGCG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAGGCGTC

22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTGTGCAC TTGAAAAACA
GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAAACAGTG AACTTTTTGT

22901 TGTA AAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCTTTTAT
ACATTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGAAAATA

22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGCGCCGT
AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACGCGGCA

23001 TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGGCAGGG
AATTTTATGTT TCCCCAAGA CGGCGCGTAG CGATACGCGG TGACCGTCCC

23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAAACTC AGGCACAACC
TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGTGTTGG

23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACCATCAC
TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGGTAGTG

23151 CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTTGGGGC
GTTGCGCAAA TCGTCCAGCC CGCGGCTATA GAACTTCAGC GTCAACCCCG

23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACTGGAAC
GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGACCTTG

23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCGGAGAT
TGATAGTCGC GGCCACCAC GTGCGACCGG TCGTGCGAGA ACAGCCTCTA

23301 CAGATCCGCG TCCAGGTCTT CCGCGTTGCT CAGGGCGAAC GGAGTCAACT
GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCAGTTGA

23351 TTGGTAGCTG CCTTCCCAA AAGGGCGCGT GCCCAGGCTT TGAGTTGCAC
AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCAACGTG

23401 TCGCACCGTA GTGGCATCAA AAGGTGACCG TGCCCGGTCT GGGCGTTAGG
AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGCAATCC

23451 ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTGAGCCT
TATGTCGCGG ACGTATTTTC GGAAC TAGAC GAATTTTCGG TGGACTCGGA

23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAAGTGATTG
AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTGACTAAC

23551 GCCGGACAGG CCGCGTCGTG CACGCGAGCAC CTTGCGTCGG TGTTGGAGAT
CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCGAGC ACAACCTCTA

23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCTTGCTAG
GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGAACGATC

23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCCATTTCA
TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGGTAAAGT

Figure 26 Y

23701 ATCACGTGCT CCTTATTTAT CATAATGCTT CCGTGTAGAC ACTTAAGCTC
 TAGTGACACGA GGAATAAATA GTATTACGAA GGCACATCTG TGAATTCGAG

23751 GCCTTCGATC TCAGCGCAGC GGTGCAGCCA CAACGCGCAG CCCGTGGGCT
 CGGAAGCTAG AGTCGCGTCG CCACGTCGGT GTTGC GCGTC GGGCACCCGA

23801 CGTGATGCTT GTAGGTCACC TCTGCAAACG ACTGCAGGTA CGCCTGCAGG
 GCACTACGAA CATCCAGTGG AGACGTTTGC TGACGTCCAT GCGGACGTCC

23851 AATCGCCCCA TCATCGTCAC AAAGGTCTTG TTGCTGGTGA AGGTCAGCTG
 TTAGCGGGGT AGTAGCAGTG TTTCCAGAAC AACGACCACT TCCAGTCGAC

23901 CAACCCGCGG TGCTCCTCGT TCAGCCAGGT CTTGCATACG GCCGCCAGAG
 GTTGGGCGCC ACGAGGAGCA AGTCGGTCCA GAACGTATGC CGGCGGTCTC

23951 CTTCCACTTG GTCAGGCAGT AGTTTGAAGT TCGCCTTTAG ATCGTTATCC
 GAAGGTGAAC CAGTCCGTCA TCAAACCTCA AGCGGAAATC TAGCAATAGG

24001 ACGTGGTACT TGTCCATCAG CGCGCGCGCA GCCTCCATGC CCTTCTCCCA
 TGCACCATGA ACAGGTAGTC GCGCGCGCGT CGGAGGTACG GGAAGAGGGT

24051 CGCAGACACG ATCGGCACAC TCAGCGGGTT CATCACCGTA ATTTCACTTT
 GCGTCTGTGC TAGCCGTGTG AGTCGCCCAA GTAGTGGCAT TAAAGTGAAG

24101 CCGCTTCGCT GGGCTCTTCC TCTTCCTCTT GCGTCCGCAT ACCACGCGCC
 GGCGAAGCGA CCCGAGAAGG AGAAGGAGAA CGCAGGCGTA TGGTGC GCGG

24151 ACTGGGTCGT CTTCAATCAG CCGCCGCACT GTGCGCTTAC CTCCTTTGCC
 TGACCCAGCA GAAGTAAGTC GGCGGCGTGA CACGCGAATG GAGGAAACGG

24201 ATGCTTGATT AGCACCGGTG GGTGCTGAA ACCCACCATT TGTAGCGCCA
 TACGAATAA TCGTGGCCAC CCAACGACTT TGGGTGGTAA ACATCGCGGT

24251 CATCTTCTCT TTCTTCCTCG CTGTCCACGA TTACCTCTGG TGATGGCGGG
 GTAGAAGAGA AAGAAGGAGC GACAGGTGCT AATGGAGACC ACTACCGCCC

24301 CGCTCGGGCT TGGGAGAAGG GCGCTTCTTT TTCTTCTTGG GCGCAATGGC
 GCGAGCCCGA ACCCTCTTCC CGCGAAGAAA AAGAAGAACC CGCGTTACCG

24351 CAAATCCGCC GCCGAGGTCG ATGGCCGCGG GCTGGGTGTG CGCGGCACCA
 GTTTAGGCGG CGGCTCCAGC TACCGGCGCC CGACCCACAC GCGCCGTGGT

24401 GCGCGTCTTG TGATGAGTCT TCCTCGTCCT CGGACTCGAT ACGCCGCTC
 CGCGCAGAAC ACTACTCAGA AGGAGCAGGA GCCTGAGCTA TGCGGCGGAG

24451 ATCCGCTTTT TTGGGGGCGC CCGGGGAGGC GCGGCGGACG GGGACGGGGA
 TAGGCGAAAA AACCCCGCG GGCCTTCCG CCGCCGCTGC CCCTGCCCTT

24501 CGACACGTCC TCCATGGTTG GGGGACGTCG CGCCGCACCG CGTCCGCGCT
 GCTGTGCAGG AGGTACCAAC CCCCTGCAGC GCGGCGTGGC GCAGGCGCGA

24551 CGGGGGTGGT TTCGCGCTGC TCCTCTTCCC GACTGGCCAT TTCCTTCTCC
 GCCCCACCA AAGCGCGACG AGGAGAAGGG CTGACCGGTA AAGGAAGAGG

24601 TATAGGCAGA AAAAGATCAT GGAGTCAGTC GAGAAGAAGG ACAGCCTAAC
 ATATCCGTCT TTTTCTAGTA CCTCAGTCAG CTCTTCTTCC TGTCGGATTG

24701 CTACCACCTT CCCCGTCGAG GCACCCCGC TTGAGGAGGA GGAAGTGATT
 GATGGTGGAA GGGGCAGCTC CGTGGGGCG AACTCCTCCT CCTTCACTAA

 24751 ATCGAGCAGG ACCCAGGTTT TGTAAGCGAA GACGACGAGG ACCGCTCAGT
 TAGCTCGTCC TGGGTCCAAA ACATTGCTT CTGCTGCTCC TGGCGAGTCA

 24801 ACCAACAGAG GATAAAAAGC AAGACCAGGA CAACGCAGAG GCAAACGAGG
 TGGTTGTCTC CTATTTTTCG TTCTGGTCCT GTTGCGTCTC CGTTTGCTCC

 24851 AACAAAGTCGG GCGGGGGGAC GAAAGGCATG GCGACTACCT AGATGTGGGA
 TTGTTACGCC CGCCCCCTG CTTTCCGTAC CGCTGATGGA TCTACACCTT

 24901 GACGACGTGC TGTTGAAGCA TCTGCAGCGC CAGTGCGCCA TTATCTGCGA
 CTGCTGCACG ACAACTTCGT AGACGTGCGG GTCACGCGGT AATAGACGCT

 24951 CGCGTTGCAA GAGCGCAGCG ATGTGCCCCT CGCCATAGCG GATGTCAGCC
 GCGCAACGTT CTCGCGTCGC TACACGGGA GCGGTATCGC CTACAGTCGG

 25001 TTGCCTACGA ACGCCACCTA TTCTCACC GC GTACCCCA CAAACGCCAA
 AACGGATGCT TGCGGTGGAT AAGAGTGGCG CGCATGGGGG GTTTGCGGTT

 25051 GAAAACGGCA CATGCGAGCC CAACCCGCGC CTCAACTTCT ACCCCGTATT
 CTTTTGCCGT GTACGCTCGG GTTGGGCGCG GAGTTGAAGA TGGGGCATAA

 25101 TGCCGTGCCA GAGGTGCTTG CCACCTATCA CATCTTTTTC CAAAACCTGCA
 ACGGCACGGT CTCCACGAAC GGTGGATAGT GTAGAAAAAG GTTTTGACGT

 25151 AGATACCCCT ATCCTGCCGT GCCAACCGCA GCCGAGCGGA CAAGCAGCTG
 TCTATGGGGA TAGGACGGCA CGGTTGGCGT CGGCTCGCCT GTTCGTGCGAC

 25201 GCCTTGCGGC AGGGCGCTGT CATACTGAT ATCGCCTCGC TCAACGAAGT
 CGGAACGCCG TCCCGCGACA GTATGGACTA TAGCGGAGCG AGTTGCTTCA

 25251 GCCAAAAATC TTTGAGGGTC TTGGACGCGA CGAGAAGCGC GCGGCAAAACG
 CGGTTTTTAG AAACCCAG AACCTGCGCT GCTCTTCGCG CGCCGTTTGC

 25301 CTCTGCAACA GGAAAACAGC GAAAATGAAA GTCACTCTGG AGTGTGGGTG
 GAGACGTTGT CCTTTTGTG CTTTTACTTT CAGTGAGACC TCACAACCAC

 25351 GAACTCGAGG GTGACAACGC GCGCCTAGCC GTACTAAAAC GCAGCATCGA
 CTTGAGCTCC CACTGTTGCG CGCGGATCGG CATGATTTTG CGTCGTAGCT

 25401 GGTCACCCAC TTTGCCTACC CGGCACTTAA CCTACCCCCC AAGGTCATGA
 CCAGTGGGTG AAACGGATGG GCCGTGAATT GGATGGGGGG TTCCAGTACT

 25451 GCACAGTCAT GAGTGAGCTG ATCGTGCGCC GTGCGCAGCC CCTGGAGAGG
 CGTGTCAGTA CTCACGCGAC TAGCACGCGG CACGCGTCGG GGACCTCTCC

 25501 GATGCAAATT TGCAAGAACA AACAGAGGAG GGCCTACCCG CAGTTGGCGA
 CTACGTTTAA ACGTTCTTGT TTGTCTCCTC CCGGATGGGC GTCAACCGCT

 25551 CGAGCAGCTA GCGCGCTGGC TTCAAACGCG CGAGCCTGCC GACTTGAGG
 GCTCGTCGAT CGCGCGACCG AAGTTTGCGC GCTCGGACGG CTGAACCTCC

Figure 26 AA

25651 TGCATGCAGC GGTTCCTTTC TGACCCGGAG ATGCAGCGCA AGCTAGAGGA
 ACGTACGTCG CCAAGAAACG ACTGGGCCTC TACGTGCGCT TCGATCTCCT

25701 AACATTGCAC TACACCTTTC GACAGGGCTA CGTACGCCAG GCCTGCAAGA
 TTGTAACGTG ATGTGGAAAG CTGTCCCGAT GCATGCGGTC CGGACGTTCT

25751 TCTCCAACGT GGAGCTCTGC AACCTGGTCT CCTACCTTGG AATTTTGCAC
 AGAGGTTGCA CCTCGAGACG TTGGACCAGA GGATGGAACC TTAAAACGTG

25801 GAAAACCGCC TTGGGCAAAA CGTGCTTCAT TCCACGCTCA AGGGCGAGGC
 CTTTTGGCGG AACCCGTTTT GCACGAAGTA AGGTGCGAGT TCCCGCTCCG

25851 GCGCCGCGAC TACGTCCGCG ACTGCGTTTA CTTATTTCTA TGCTACACCT
 CGCGGCGCTG ATGCAGGCGC TGACGCAAAAT GAATAAAGAT ACGATGTGGA

25901 GGCAGACGGC CATGGGCGTT TGGCAGCAGT GCTTGGAGGA GTGCAACCTC
 CCGTCTGCCG GTACCCGCAA ACCGTCGTCA CGAACCTCCT CACGTTGGAG

25951 AAGGAGCTGC AGAAACTGCT AAAGCAAAAC TTGAAGGACC TATGGACGGC
 TTCCTCGACG TCTTTGACGA TTTCGTTTTG AACTTCCTGG ATACCTGCCG

26001 CTTCAACGAG CGCTCCGTGG CCGCGCACCT GGCGGACATC ATTTTCCCCG
 GAAGTTGCTC GCGAGGCACC GGC GCGTGGA CCGCCTGTAG TAAAAGGGGC

26051 AACGCCTGCT TAAAACCTTG CAACAGGGTC TGCCAGACTT CACCAGTCAA
 TTGCGGACGA ATTTTGGGAC GTTGTTCCAG ACGGTCTGAA GTGGTCAGTT

26101 AGCATGTTGC AGAACTTTAG GAACTTTATC CTAGAGCGCT CAGGAATCTT
 TCGTACAACG TCTTGAAATC CTTGAAATAG GATCTCGCGA GTCCTTAGAA

26151 GCCCCGCCACC TGCTGTGCAC TTCCTAGCGA CTTTGTGCCC ATTAAGTACC
 CGGGCGGTGG ACGACACGTG AAGGATCGCT GAAACACGGG TAATTCATGG

26201 GCGAATGCCC TCCGCCGCTT TGGGGCCACT GCTACCTTCT GCAGCTAGCC
 CGCTTACGGG AGGCGGCGAA ACCCCGGTGA CGATGGAAGA CGTCGATCGG

26251 AACTACCTTG CCTACCACTC TGACATAATG GAAGACGTGA GCGGTGACGG
 TTGATGGAAC GGATGGTGAG ACTGTATTAC CTTCTGCACT CGCCACTGCC

26301 TCTACTGGAG TGTCACGTGC GCTGCAACCT ATGCACCCCG CACCGCTCCC
 AGATGACCTC ACAGTGACAG CGACGTTGGA TACGTGGGGC GTGGCGAGGG

26351 TGTTTTGCAA TTCGCAGCTG CTTAACGAAA GTCAAATTAT CGGTACCTTT
 ACCAAACGTT AAGCGTCGAC GAATTGCTTT CAGTTTAATA GCCATGGAAA

26401 GAGCTGCAGG GTCCCTCGCC TGACGAAAAG TCCGCGGCTC CGGGGTTGAA
 CTCGACGTCC CAGGGAGCGG ACTGCTTTTC AGGCGCCGAG GCCCCAACTT

26451 ACTCACTCCG GGGCTGTGGA CGTCGGCTTA CCTTCGCAAA TTTGTACCTG
 TGAGTGAGGC CCCGACACCT GCAGCCGAAT GGAAGCGTTT AAACATGGAC

26501 AGGACTACCA CGCCCACGAG ATTAGGTTCT ACGAAGACCA ATCCCCCCCC
 TCCTGATGGT GCGGGTGCTC TAATCCAAGA TGCTTCTGGT TAGGGCGGGC

Figure 26 AB

| | | | | | |
|-------|--------------------------|--------------------------|---------------------------|---------------------------|--------------------------|
| 26551 | CCTAATGCGG GGATTACGCC | AGCTTACCGC TCGAATGGCG | CTGCGTCATT GACGCAGTAA | ACCCAGGGCC TGGGTCCC | ACATTCTTGG TGTAAGAACC |
| 26601 | CCAATTGCAA GGTTAACGTT | GCCATCAACA CGGTAGTTGT | AAGCCCGCCA TTCGGGCGGT | AGAGTTTCTG TCTCAAAGAC | CTACGAAAGG GATGCTTTCC |
| 26651 | GACGGGGGGT CTGCCCCCA | TTACTTGGAC AATGAACCTG | CCCCAGTCCG GGGGTCAGGC | GCGAGGAGCT CGCTCCTCGA | CAACCCAATC GTTGGGTTAG |
| 26701 | CCCCCGCCGC GGGGCGGCG | CGCAGCCCTA GCGTCGGGAT | TCAGCAGCAG AGTCGTCGTC | CCGCGGGCCC GGCGCCCGG | TTGCTTCCCA AACGAAGGGT |
| 26751 | GGATGGCACC CCTACCGTGG | CAAAAAGAAG GTTTTTCTTC | CTGCAGCTGC GACGTCGACG | CGCCGCCACC GCGGCGGTGG | CACGGACGAG GTGCTTGCTC |
| 26801 | GAGGAATACT CTCCTTATGA | GGGACAGTCA CCCTGTCAGT | GGCAGAGGAG CCGTCTCCTC | GTTTTGGACG CAAAACCTGC | AGGAGGAGGA TCCTCCTCCT |
| 26851 | GGACATGATG CCTGTACTAC | GAAGACTGGG CTTCTGACCC | AGAGCCTAGA TCTCGGATCT | CGAGGAAGCT GCTCCTTCGA | TCCGAGGTCC AGGCTCCAGC |
| 26901 | AAGAGGTGTC TTCTCCACAG | AGACGAAACA TCTGCTTTGT | CCGTCACCCCT GGCAGTGGGA | CGGTGCGCATT GCCAGCGTAA | CCCCTCGCCG GGGGAGCGGC |
| 26951 | GCGCCCCAGA CGCGGGGTCT | AATCGGCAAC TTAGCCGTTG | CGGTTCCAGC GCCAAGGTCG | ATGGCTACAA TACCGATGTT | CCTCCGCTCC GGAGGCGAGG |
| 27001 | TCAGGCGCCG AGTCCGCGGC | CCGGCACTGC GGCCGTGACG | CCGTTGCGCCG GGCAAGCGGC | ACCCAACCGT TGGGTGGCA | AGATGGGACA TCTACCCTGT |
| 27051 | CCACTGGAAC GGTGACCCTG | CAGGGCCGGT GTCCCGGCCA | AAGTCCAAGC TTCAGGTTTCG | AGCCGCCGCC TCGGCGGCGG | GTTAGCCCAA CAATCGGGTT |
| 27101 | GAGCAACAAC CTCGTTGTG | AGCGCCAAGG TCGCGGTTCC | CTACCGCTCA GATGGCGAGT | TGGCGCGGGC ACCGCGCCCG | ACAAGAACGC TGTTCTTGCG |
| 27151 | CATAGTTGCT GTATCAACGA | TGCTTGCAAG ACGAACGTTT | ACTGTGGGGG TGACACCCCC | CAACATCTCC GTTGTAGAGG | TTGCCCCGCC AAGCGGGCGG |
| 27201 | GCTTTCTTCT CGAAAGAAGA | CTACCATCAC GATGGTAGTG | GGCGTGGCCT CCGCACCGGA | TCCCCCGTAA AGGGGGCATT | CATCCTGCAT GTAGGACGTA |
| 27251 | TACTACCGTC ATGATGGCAG | ATCTCTACAG TAGAGATGTC | CCCATACTGC GGGTATGACG | ACCGGCGGCA TGGCCGCCGT | GCGGCAGCAA CGCCGTCGTT |
| 27301 | CAGCAGCGGC GTCGTCGCCG | CACACAGAAG GTGTGTCCTC | CAAAGGCGAC GTTTCCGCTG | CGGATAGCAA GCCTATCGTT | GACTCTGACA CTGAGACTGT |
| 27351 | AAGCCCAAGA TTCGGGTTCT | AATCCACAGC TTAGGTGTCG | GGCGGCAGCA CCGCCGTCGT | GCAGGAGGAG CGTCCTCCTC | GAGCGCTGCG CTCGCGACGC |
| 27401 | TCTGGCGCCC AGACCGCGGG | AACGAACCCG TTGCTTGGGC | TATCGACCCG ATAGCTGGGC | CGAGCTTAGA GCTCGAATCT | AACAGGATTT TTGTCTTAA |
| 27451 | TTCCCACTCT AAGGGTGAGA | GTATGCTATA CATACGATAT | TTCAACAGA AAAGTTGTCT | GCAGGGGCCA CGTCCCCGGT | AGAACAAGAG TCTTGTCTCT |

Figure 26. AC

27551 TCACAAAAGC GAAGATCAGC TTCGGCGCAC GCTGGAAGAC GCGGAGGCTC
 AGTGTTTTTCG CTTCTAGTCG AAGCCGCGTG CGACCTTCTG CGCCTCCGAG

27601 TCTTCAGTAA ATACTGCGCG CTGACTCTTA AGGACTAGTT TCGCGCCCTT
 AGAAGTCATT TATGACGCGC GACTGAGAAT TCCTGATCAA AGCGCGGGAA

27651 TCTCAAATTT AAGCGCGAAA ACTACGTCAT CTCCAGCGGC CACACCCGGC
 AGAGTTTAAA TTCGCGCTTT TGATGCAGTA GAGGTCGCCG GTGTGGGCCG

27701 GCCAGCACCT GTTGTGAGCG CCATTATGAG CAAGGAAATT CCCACGCCCT
 CGGTGCTGGA CAACAGTCGC GGTAATACTC GTTCCTTTAA GGGTGCGGGA

27751 ACATGTGGAG TTACCAGCCA CAAATGGGAC TTGCGGCTGG AGCTGCCCAA
 TGTACACCTC AATGGTCGGT GTTTACCCTG AACGCCGACC TCGACGGGTT

27801 GACTACTCAA CCCGAATAAA CTACATGAGC GCGGGACCCC ACATGATATC
 CTGATGAGTT GGGCTTATTT GATGTACTCG CGCCCTGGGG TGTACTATAG

27851 CCGGGTCAAC GGAATACGCG CCCACCGAAA CCGAATTCTC CTGGAACAGG
 GGCCAGTTG CCTTATGCGC GGGTGGCTTT GGCTTAAGAG GACCTTGTC

27901 CGGCTATTAC CACCACACCT CGTAATAACC TTAATCCCCG TAGTTGGCCC
 GCCGATAATG GTGGTGTGGA GCATTATTGG AATTAGGGGC ATCAACCGGG

27951 GCTGCCCTGG TGTACCAGGA AAGTCCCGCT CCCACCACTG TGGTACTTCC
 CGACGGGACC ACATGGTCCT TTCAGGGCGA GGGTGGTGAC ACCATGAAGG

28001 CAGAGACGCC CAGGCCGAAG TTCAGATGAC TAACTCAGGG GCGCAGCTTG
 GTCTCTGCGG GTCCGGCTTC AAGTCTACTG ATTGAGTCCC CGCGTCGAAC

28051 CGGGCGGCTT TCGTCACAGG GTGCGGTCGC CCGGGCAGGG TATAACTCAC
 GCGCGCCGAA AGCAGTGTC CACGCCAGCG GGCCCGTCCC ATATTGAGTG

28101 CTGACAATCA GAGGGCGAGG TATTCAGCTC AACGACGAGT CGGTGAGCTC
 GACTGTTAGT CTCCCGCTCC ATAAGTCGAG TTGCTGCTCA GCCACTCGAG

28151 CTCGCTTGGT CTCCGTCCGG ACGGGACATT TCAGATCGGC GCGCGCGGCC
 GAGCGAACCA GAGGCAGGCC TGCCCTGTAA AGTCTAGCCG CCGCGGCCGG

28201 GCTCTTCATT CACGCCTCGT CAGGCAATCC TAACTCTGCA GACCTCGTCC
 CGAGAAGTAA GTGCGGAGCA GTCCGTTAGG ATTGAGACGT CTGGAGCAGG

28251 TCTGAGCCGC GCTCTGGAGG CATTGGAAC TCGCAATTTA TTGAGGAGTT
 AGACTCGGCG CGAGACCTCC GTAACCTTGA GACGTTAAAT AACTCCTCAA

28301 TGTGCCATCG GTCTACTTTA ACCCCTTCTC GGGACCTCCC GGCCACTATC
 ACACGGTAGC CAGATGAAAT TGGGGAAGAG CCCTGGAGGG CCGGTGATAG

28351 CGGATCAATT TATTCCTAAC TTTGACGCGG TAAAGGACTC GGCGGACGGC
 GCCTAGTTAA ATAAGGATTG AAAGTGCGCC ATTTCTCTGAG CCGCCTGCCG

28401 TACGACTGAA TGTTAAGTGG AGAGGCAGAG CAACTGCGCC TGAAACACCT
 ATGCTGACTT ACAATTCACC TCTCCGTCTC GTTGACGCGG ACTTTGTGGA

Figure 26 AD

28451 GGTCCACTGT CGCCGCCACA AGTGCTTTGC CCGCGACTCC GGTGAGTTTT
 CCAGGTGACA GCGGCGGTGT TCACGAAACG GCGGCTGAGG CCACTCAAAA

 28501 GCTACTTTGA ATTGCCCCGAG GATCATATCG AGGGCCCCGGC GCACGGCGTC
 CGATGAAACT TAACGGGCTC CTAGTATAGC TCCCGGGCCG CGTGCCGCAG

 28551 CGGCTTACCG CCCAGGGAGA GCTTGCCCGT AGCCTGATTG GGGAGTTTAC
 GCCGAATGGC GGGTCCCTCT CGAACGGGCA TCGGACTAAG CCCTCAAATG

 28601 CCAGCGCCCC CTGCTAGTTG AGCGGGACAG GGGACCCTGT GTTCTCACTG
 GGTGCGGGG GACGATCAAC TCGCCCTGTC CCCTGGGACA CAAGAGTGAC

 28651 TGATTTGCAA CTGTCCTAAC CCTGGATTAC ATCAAGATCT TTGTTGCCAT
 ACTAAACGTT GACAGGATTG GGACCTAATG TAGTTCTAGA AACAACGGTA

 28701 CTCTGTGCTG AGTATAATAA ATACAGAAAT TAAAATATAC TGGGGCTCCT
 GAGACACGAC TCATATTATT TATGTCTTTA ATTTTATATG ACCCCGAGGA

 28751 ATCGCCATCC TGTAACGCC ACCGTCTTCA CCCGCCCAAG CAAACCAAGG
 TAGCGGTAGG ACATTTGCGG TGGCAGAAGT GGGCGGGTTC GTTTGGTTCC

 28801 CGAACCTTAC CTGGTACTTT TAACATCTCT CCCTCTGTGA TTTACAACAG
 GCTTGGAATG GACCATGAAA ATTGTAGAGA GGGAGACACT AAATGTTGTC

 28851 TTTCAACCCA GACGGAGTGA GTCTACGAGA GAACCTCTCC GAGCTCAGCT
 AAAGTTGGGT CTGCCTCACT CAGATGCTCT CTTGGAGAGG CTCGAGTCGA

 28901 ACTCCATCAG AAAAAACACC ACCCTCCTTA CCTGCCGGGA ACGTACGAGT
 TGAGGTAGTC TTTTTTGTGG TGGGAGGAAT GGACGGCCCT TGCATGCTCA

 28951 GCGTCACCGG CCGCTGCACC ACACCTACCG CCTGACCGTA AACCAGACTT
 CGCAGTGGCC GGCGACGTGG TGTGGATGGC GGACTGGCAT TTGGTCTGAA

 29001 TTTCCGGACA GACCTCAATA ACTCTGTTTA CCAGAACAGG AGGTGAGCTT
 AAAGGCCTGT CTGGAGTTAT TGAGACAAAT GGTCTTGTCC TCCACTCGAA

 29051 AGAAAACCTT TAGGGTATTA GGCCAAAGGC GCAGCTACTG TGGGGTTTAT
 TCTTTTGGGA ATCCCATAAAT CCGGTTTCCG CGTCGATGAC ACCCCAAATA

 29101 GAACAATTCA AGCAACTCTA CGGGCTATTG TAATTCAGGT TTCTCTAGAA
 CTTGTTAAGT TCGTTGAGAT GCCCGATAAG ATTAAGTCCA AAGAGATCTT

 29151 TCGGGGTTGG GGTATTCTC TGTCTTGTA TTCTCTTTAT TCTTATACTA
 AGCCCCAACC CCAATAAGAG ACAGAACT AAGAGAAATA AGAATATGAT

 29201 ACGCTTCTCT GCCTAAGGCT CGCCGCCTGC TGTGTGCACA TTTGCATTTA
 TGCGAAGAGA CGGATTCCGA GCGGCGGACG ACACACGTGT AAACGTAAAT

 29251 TTGTCAGCTT TTTAAACGCT GGGGTCGCCA CCCAAGATGA TTAGGTACAT
 AACAGTCGAA AAATTTGCGA CCCAGCGGT GGGTTCTACT AATCCATGTA

 29301 AATCCTAGGT TTAATCAGCT TTGCGTCAGC CCACGGTACC ACCCAAAGG
 TTAGGATCCA AATGAGTGGG AACGCAGTCG GGTGCCATGG TGGGTTTTCC

 29351 TGGATTTTAA GGAGCCAGCC TGTAATGTGA CATTGCGAGC TGAAGCTAAT
 ACCTAAAATT CCTCGGTCGG ACATTACAAT GTAAGCGTCG ACTTCGATTA

Figure 26 AE

29451 TCGCCACAAA AACAAAATTG GCAAGTATGC TGTATTATGCT ATTTGGCAGC
 AGCGGTGTTT TTGTTTTAAC CGTTCATACG ACAAATACGA TAAACCGTCG

29501 CAGGTGACAC TACAGAGTAT AATGTTACAG TTTTCCAGGG TAAAAGTCAT
 GTCCACTGTG ATGTCTCATA TTACAATGTC AAAAGGTCCC ATTTTCAGTA

29551 AAAACTTTTA TGTATACTTT TCCATTTTAT GAAATGTGCG ACATTACCAT
 TTTTGAAAAT ACATATGAAA AGGTAAAATA CTTTACACGC TGTAATGGTA

29601 GTACATGAGC AAACAGTATA AGTTGTGGCC CCCACAAAAT TGTGTGGAAA
 CATGTACTCG TTTGTCATAT TCAACACCGG GGGTGTTTTA ACACACCTTT

29651 ACACTGGCAC TTTCTGCTGC ACTGCTATGC TAATTACAGT GCTCGCTTTG
 TGTGACCGTG AAAGACGACG TGACGATACG ATTAATGTCA CGAGCGAAAC

29701 GTCTGTACCC TACTCTATAT TAAATACAAA AGCAGACGCA GCTTTATTGA
 CAGACATGGG ATGAGATATA ATTTATGTTT TCGTCTGCGT CGAAATAACT

29751 GGAAAAGAAA ATGCCTTAAT TTAATAAGTT ACAAAGCTAA TGTCACCACT
 CCTTTCTTT TACGGAATTA AATGATTCAA TGTTTCGATT ACAGTGGTGA

29801 AACTGCTTTA CTCGCTGCTT GCAAAACAAA TTCAAAAAGT TAGCATTATA
 TTGACGAAAT GAGCGACGAA CGTTTTGTTT AAGTTTTTCA ATCGTAATAT

29851 ATTAGAATAG GATTTAAACC CCCC GGTCAT TTCCTGCTCA ATACCATTCC
 TAATCTTATC CTAAATTTGG GGGGCCAGTA AAGGACGAGT TATGGTAAGG

29901 CCTGAACAAT TGACTCTATG TGGGATATGC TCCAGCGCTA CAACCTTGAA
 GGACTTGTTA ACTGAGATAC ACCCTATACG AGGTCGCGAT GTTGGAACCT

29951 GTCAGGCTTC CTGGATGTCA GCATCTGACT TTGGCCAGCA CCTGTCCCGC
 CAGTCCGAAG GACCTACAGT CGTAGACTGA AACCGGTCGT GGACAGGGCG

30001 GGATTTGTTC CAGTCCAACCT ACAGCGACCC ACCCTAACAG AGATGACCAA
 CCTAAACAAG GTCAGGTTGA TGTCGCTGGG TGGGATTGTC TCTACTGGTT

30051 CACAACCAAC GCGGCCGCCG CTACCGGACT TACATCTACC ACAAATACAC
 GTGTTGGTTG CGCCGGCGGC GATGGCCTGA ATGTAGATGG TGTTTATGTG

30101 CCCAAGTTTC TGCCTTTGTC AATAACTGGG ATAACCTGGG CATGTGGTGG
 GGGTTCAAAG ACGGAAACAG TTATTGACCC TATTGAACCC GTACACCACC

30151 TTCTCCATAG CGCTTATGTT TGTATGCCTT ATTATTATGT GGCTCATCTG
 AAGAGGTATC GCGAATACAA ACATACGGAA TAATAATACA CCGAGTAGAC

30201 CTGCCTAAAG CGCAAACGCG CCCGACCACC CATCTATAGT CCCATCATTG
 GACGGATTTT GCGTTTGCGC GGGCTGGTGG GTAGATATCA GGGTAGTAAC

30251 TGCTACACCC AAACAATGAT GGAATCCATA GATTGGACGG ACTGAAACAC
 ACGATGTGGG TTTGTTACTA CCTTAGGTAT CTAACCTGCC TGACTTTGTG

30301 ATGTTCTTTT CTCTTACAGT ATGATTAAAT GAGACATGAT TCCTCGAGTT
 TACAAGAAAA GAGAATGTCA TACTAATTTA CTCTGTACTA AGGAGCTCAA

Figure 26 AF

30401 TCGGGTTTCT CACATCGAAG TAGACTGCAT TCCAGCCTTC ACAGTCTATT
 ACGCCAAAGA GTGTAGCTTC ATCTGACGTA AGGTCGGAAG TGTCAGATAA

 30451 TGCTTTACGG ATTTGTCACC CTCACGCTCA TCTGCAGCCT CATCACTGTG
 ACGAAATGCC TAAACAGTGG GAGTGCAGT AGACGTCGGA GTAGTGACAC

 30501 GTCATCGCCT TTATCCAGTG CATTGACTGG GTCTGTGTGC GCTTTGCATA
 CAGTAGCGGA AATAGGTCAC GTAACGTACC CAGACACACG CGAAACGTAT

 30551 TCTCAGACAC CATCCCCAGT ACAGGGACAG GACTATAGCT GAGCTTCTTA
 AGAGTCTGTG GTAGGGGTCA TGTCCTGTG CTGATATCGA CTCGAAGAAT

 30601 GAATTCTTTA ATTATGAAAT TTACTGTGAC TTTTCTGCTG ATTATTTGCA
 CTTAAGAAAT TAATACTTTA AATGACACTG AAAAGACGAC TAATAAACGT

 30651 CCCTATCTGC GTTTTGTTC CCGACCTCCA AGCCTCAAAG ACATATATCA
 GGGATAGACG CAAAACAAGG GGCTGGAGGT TCGGAGTTTC TGTATATAGT

 30701 TGCAGATTCA CTCGTATATG GAATATTCCA AGTTGCTACA ATGAAAAAAG
 ACGTCTAAGT GAGCATATAC CTTATAAGGT TCAACGATGT TACTTTTTTC

 30751 CGATCTTTCC GAAGCCTGGT TATATGCAAT CATCTCTGTT ATGGTGTCTT
 GCTAGAAAGG CTTTCGGACCA ATATACGTTA GTAGAGACAA TACCACAAGA

 30801 GCAGTACCAT CTTAGCCCTA GCTATATATC CCTACCTTGA CATTGGCTGG
 CGTCATGGTA GAATCGGGAT CGATATATAG GGATGGAAGT GTAACCGACC

 30851 AACGCAATAG ATGCCATGAA CCACCCAACT TTCCCCGCGC CCGCTATGCT
 TTGCGTTATC TACGGTACTT GGTGGGTGA AAGGGGCGCG GCGGATACGA

 30901 TCCACTGCAA CAAGTTGTTG CCGGCGGCTT TGTCCCAGCC AATCAGCCTC
 AGGTGACGTT GTTCAACAAC GGCCGCCGAA ACAGGGTCGG TTAGTCGGAG

 30951 GCCCACCTTC TCCCACCCCC ACTGAAATCA GCTACTTTAA TCTAACAGGA
 CGGGTGGAAG AGGGTGGGGG TGACTTTAGT CGATGAAATT AGATTGTCTT

 31001 GGAGATGACT GACACCCTAG ATCTAGAAAT GGACGGAATT ATTACAGAGC
 CCTCTACTGA CTGTGGGATC TAGATCTTTA CCTGCCTTAA TAATGTCTCG

 31051 AGCGCCTGCT AGAAAGACGC AGGGCAGCGG CCGAGCAACA GCGCATGAAT
 TCGCGGACGA TCTTCTGCG TCCCGTCGCC GGCTCGTTGT CGCGTACTTA

 31101 CAAGAGCTCC AAGACATGGT TAACTTGCAC CAGTGCAAAA GGGGTATCTT
 GTTCTCGAGG TTCTGTACCA ATTGAACGTG GTCACGTTTT CCCCATAGAA

 31151 TTGTCTCGTA AAGCAGGCCA AAGTCACCTA CGACAGTAAT ACCACCGGAC
 AACAGAGCAT TTCGTCCGGT TTCAGTGGAT GCTGTCATTA TGGTGGCCTG

 31201 ACCGCCTTAG CTACAAGTTG CCAACCAAGC GTCAGAAATT GGTGGTCATG
 TGGCGGAATC GATGTTCAAC GGTGGTTTCG CAGTCTTTAA CCACCACTAC

 31251 GTGGGAGAAA AGCCCATAC CATAACTCAG CACTCGGTAG AAACCGAAGG
 CACCTCTTT TCGGGTAATG GTATTGAGTC GTGAGCCATC TTTGGCTTCC

Figure 26 AG

31351 AGACCTGTG CGGTCTCAAA GATCTTATTC CCTTTAACTA ATAAAAAAAA
 TCTGGGACAC GCCAGAGTTT CTAGAATAAG GGAAATTGAT TATTTTTTTT

 31401 ATAATAAAGC ATCACTTACT TAAAATCAGT TAGCAAATTT CTGTCCAGTT
 TATTATTTTCG TAGTGAATGA ATTTTAGTCA ATCGTTTAAA GACAGGTCAA

 31451 TATTCAGCAG CACCTCCTTG CCCTCCTCCC AGCTCTGGTA TTGCAGCTTC
 ATAAGTCGTC GTGGAGGAAC GGGAGGAGGG TCGAGACCAT AACGTCGAAG

 31501 CTCCTGGCTG CAAACTTTCT CCACAATCTA AATGGAATGT CAGTTTCCTC
 GAGGACCGAC GTTTGAAAGA GGTGTTAGAT TTACCTTACA GTCAAAGGAG

 31551 CTGTTCTGT CCATCCGCAC CCACTATCTT CATGTTGTTG CAGATGAAGC
 GACAAGGACA GGTAGGCGTG GGTGATAGAA GTACAACAAC GTCTACTTCG

 31601 GCGCAAGACC GTCTGAAGAT ACCTTCAACC CCGTGTATCC ATATGACACG
 CGCGTTCTGG CAGACTTCTA TGGAAGTTGG GGCACATAGG TATACTGTGC

 31651 GAAACCGGTC CTCCAACGTG GCCTTTTCTT ACTCCTCCCT TTGTATCCCC
 CTTTGGCCAG GAGGTTGACA CGGAAAAGAA TGAGGAGGGA AACATAGGGG

 31701 CAATGGGTTT CAAGAGAGTC CCCCTGGGGT ACTCTCTTTG CGCCTATCCG
 GTTACCCAAA GTTCTCTCAG GGGGACCCCA TGAGAGAAAC GCGGATAGGC

 31751 AACCTCTAGT TACCTCCAAT GGCATGCTTG CGCTCAAAT GGGCAACGGC
 TTGGAGATCA ATGGAGGTTA CCGTACGAAC GCGAGTTTAA CCCGTTGCCG

 31801 CTCTCTCTGG ACGAGGCCGG CAACCTTACC TCCCAAATG TAACCACTGT
 GAGAGAGACC TGCTCCGGCC GTTGGGAATGG AGGGTTTTAC ATTGGTGACA

 31851 GAGCCACCT CTCAAAAAA CCAAGTCAAA CATAAACCTG GAAATATCTG
 CTCGGGTGGA GAGTTTTTTT GGTTCAGTTT GTATTTGGAC CTTTATAGAC

 31901 CACCCCTCAC AGTTACCTCA GAAGCCCTAA CTGTGGCTGC CGCCGCACCT
 GTGGGGAGTG TCAATGGAGT CTTCCGGATT GACACCGACG GCGGCGTGGA

 31951 CTAATGGTCG CGGGCAACAC ACTCACCATG CAATCACAGG CCCCCTAAC
 GATTACCAGC GCCCGTTGTG TGAGTGGTAC GTTAGTGTCC GGGGCGATTG

 32001 CGTGACGAC TCCAAACTTA GCATTGCCAC CCAAGGACCC CTCACAGTGT
 GCACGTGCTG AGGTTTGAAT CGTAACGGTG GGTTCCTGGG GAGTGTACA

 32051 CAGAAGGAAA GCTAGCCCTG CAAACATCAG GCCCCCTCAC CACCACCGAT
 GTCTTCCTTT CGATCGGGAC GTTTGTAGTC CGGGGGAGTG GTGGTGGCTA

 32101 AGCAGTACCC TTAATATCAC TGCCTCACCC CCTCTAACTA CTGCCACTGG
 TCGTCATGGG AATGATAGTG ACGGAGTGGG GGAGATTGAT GACGGTGACC

 32151 TAGCTTGGGC ATTGACTTGA AAGAGCCCAT TTATACACAA AATGGAAAA
 ATCGAACCCG TAACTGAACT TTCTCGGGTA AATATGTGTT TTACCTTTTG

 32201 TAGGACTAAA GTACGGGGCT CCTTTGCATG TAACAGACGA CCTAAACACT
 ATCCTGATTT CATGCCCCGA GGAAACGTAC ATTGTCTGCT GGATTTGTGA

Figure 26 AH

32301 AACTAAAGTT ACTGGAGCCT TGGGTTTTGA TTCACAAGGC AATATGCAAC
TTGATTTCAA TGACCTCGGA ACCCAAAACT AAGTGTTCG TTATACGTTG

32351 TTAATGTAGC AGGAGGACTA AGGATTGATT CTCAAAACAG ACGCCTTATA
AATTACATCG TCCTCCTGAT TCCTAACTAA GAGTTTTGTC TCGGAATAT

32401 CTTGATGTTA GTTATCCGTT TGATGCTCAA AACCAACTAA ATCTAAGACT
GAACTACAAT CAATAGGCAA ACTACGAGTT TTGGTTGATT TAGATTCTGA

32451 AGGACAGGGC CCTCTTTTTTA TAAACTCAGC CCACAACCTG GATATTAACCT
TCCTGTCCCG GGAGAAAAAT ATTTGAGTCG GGTGTTGAAC CTATAATTGA

32501 ACAACAAAGG CCTTTACTTG TTTACAGCTT CAAACAATTC CAAAAAGCTT
TGTTGTTTCC GGAAATGAAC AAATGTCGAA GTTTGTTAAG GTTTTTCGAA

32551 GAGGTTAACC TAAGCACTGC CAAGGGGTTG ATGTTTGACG CTACAGCCAT
CTCCAATTGG ATTCGTGACG GTTCCCCAAC TACAACTGC GATGTCGGTA

32601 AGCCATTAAT GCAGGAGATG GGCTTGAATT TGGTTCACCT AATGCACCAA
TCGGTAATTA CGTCCTCTAC CCGAACTTAA ACCAAGTGGA TTACGTGGTT

32651 ACACAAATCC CCTCAAAACA AAAATTGGCC ATGGCCTAGA ATTTGATTCA
TGTGTTTAGG GGAGTTTTGT TTTTAACCGG TACCGGATCT TAACTAAGT

32701 AACAAAGGCTA TGGTTCCTAA ACTAGGAACT GGCCTTAGTT TTGACAGCAC
TTGTTCCGAT ACCAAGGATT TGATCCTTGA CCGGAATCAA AACTGTCGTG

32751 AGGTGCCATT ACAGTAGGAA ACAAAAATAA TGATAAGCTA ACTTTGTGGA
TCCACGGTAA TGTCATCCTT TGTTTTTATT ACTATTGATG TGAAACACCT

32801 CCACACCAGC TCCATCTCCT AACTGTAGAC TAAATGCAGA GAAAGATGCT
GGTGTGGTCG AGGTAGAGGA TTGACATCTG ATTTACGTCT CTTTCTACGA

32851 AAACCTCACTT TGGTCTTAAC AAAATGTGGC AGTCAAATAC TTGCTACAGT
TTTGAGTGAA ACCAGAATTG TTTTACACCG TCAGTTTATG AACGATGTCA

32901 TTCAGTTTTG GCTGTTAAAG GCAGTTTGGC TCCAATATCT GGAACAGTTC
AAGTCAAAAC CGACAATTTC CGTCAAACCG AGGTTATAGA CTTGTGCAAG

32951 AAAGTGCTCA TCTTATTATA AGATTGACG AAAATGGAGT GCTACTAAAC
TTTCACGAGT AGAATAATAT TCTAACTGC TTTTACCTCA CGATGATTTG

33001 AATTCCTTCC TGGACCCAGA ATATTGGAAC TTTAGAAATG GAGATCTTAC
TTAAGGAAGG ACCTGGGTCT TATAACCTTG AAATCTTTAC CTCTAGAATG

33051 TGAAGGCACA GCCTATACAA ACGCTGTTGG ATTTATGCCT AACCTATCAG
ACTTCCGTGT CGGATATGTT TGCGACAACC TAAATACGGA TTGGATAGTC

33101 CTTATCCAAA ATCTCACGGT AAAACTGCCA AAAGTAACAT TGTCAGTCAA
GAATAGGTTT TAGAGTGCCA TTTTGACGGT TTTCAATTGA ACAGTCAGTT

33151 GTTTACTTAA ACGGAGACAA AACTAAACCT GTAACACTAA CCATTACACT
CAAATGAATT TGCTCTGTT TTGATTTGGA CATTGTGATT GGTAATGTGA

Figure 26 AI

33251 CATTTCATG GGACTGGTCT GGCCACAAC ACATTAATGA AATATTTGCC
 GTAAAAGTAC CCTGACCAGA CCGGTGTTGA TGTAAATTACT TTATAAACGG

33301 ACATCCTCTT ACACTTTTTC ATACATTGCC CAAGAATAAA GAATCGTTTG
 TGTAGGAGAA TGTGAAAAAG TATGTAACGG GTTCTTATTT CTTAGCAAAC

33351 TGTATGTTT CAACGTGTTT ATTTTCAAT TGCAGAAAAT TTCAAGTCAT
 ACAATACAAA GTTGACAAA TAAAAAGTTA ACGTCTTTTA AAGTTCAGTA

33401 TTTTCATTCA GTAGTATAGC CCCACCACCA CATAGCTTAT ACAGATCACC
 AAAAGTAAGT CATCATATCG GGGTGCTGGT GTATCGAATA TGTCTAGTGG

33451 GTACCTTAAT CAAACTCACA GAACCCTAGT ATTCAACCTG CCACCTCCCT
 CATGGAATTA GTTTGAGTGT CTTGGGATCA TAAGTTGGAC GGTGGAGGGA

33501 CCCAACACAC AGAGTACACA GTCCTTTCTC CCCGGCTGGC CTTAAAAAGC
 GGGTTGTGTG TCTCATGTGT CAGGAAAGAG GGGCCGACCG GAATTTTTTCG

33551 ATCATATCAT GGGTAACAGA CATATTCTTA GGTGTTATAT TCCACACGGT
 TAGTATAGTA CCCATTGTCT GTATAAGAAAT CCACAATATA AGGTGTGCCA

33601 TTCCTGTCGA GCCAAACGCT CATCAGTGAT ATTAATAAAC TCCCCGGGCA
 AAGGACAGCT CGGTTTGCGA GTAGTCACTA TAATTATTTG AGGGGCCCGT

33651 GCTCACTTAA GTTCATGTCG CTGTCCAGCT GCTGAGCCAC AGGCTGCTGT
 CGAGTGAATT CAAGTACAGC GACAGGTCGA CGACTCGGTG TCCGACGACA

33701 CCAACTTGCG GTTGCTTAAC GGGCGGCGAA GGAGAAGTCC ACGCCTACAT
 GGTGGAACGC CAACGAATTG CCCGCCGCTT CCTCTTCAGG TGCGGATGTA

33751 GGGGGTAGAG TCATAATCGT GCATCAGGAT AGGGCGGTGG TGCTGCAGCA
 CCCCCATCTC AGTATTAGCA CGTAGTCCTA TCCCGCCACC ACGACGTCGT

33801 GCGCGCGAAT AAACCTGCTGC CGCCGCCGCT CCGTCCTGCA GGAATACAAC
 CGCGCGCTTA TTTGACGACG GCGGCGGCGA GGCAGGACGT CCTTATGTTG

33851 ATGGCAGTGG TCTCCTCAGC GATGATTCGC ACCGCCCGCA GCATAAGGCG
 TACCGTCACC AGAGGAGTCG CTACTAAGCG TGGCGGGCGT CGTATTCCGC

33901 CCTTGTCCTC CGGGCACAGC AGCGCACCCT GATCTCACTT AAATCAGCAC
 GGAACAGGAG GCGCGTGTG TCGCGTGGGA CTAGAGTGAA TTTAGTCGTG

33951 AGTAACTGCA GCACAGCACC ACAATATTGT TCAAAATCCC ACAGTGCAAG
 TCATTGACGT CGTGTCTGTTG TGTATAACA AGTTTTAGGG TGTCACGTTC

34001 GCGCTGTATC CAAAGCTCAT GGCGGGGACC ACAGAACCCA CGTGGCCATC
 CGCGACATAG GTTTCGAGTA CCGCCCCCTGG TGTCTTGGGT GCACCGGTAG

34051 ATACCACAAG CGCAGGTAGA TTAAGTGGCG ACCCCTCATA AACACGCTGG
 TATGGTGTTC GCGTCCATCT AATTCAACGC TGGGGAGTAT TTGTGCGACC

34101 ACATAAACAT TACCTCTTTT GGCATGTTGT AATTCACCAC CTCCCGGTAC
 TGTATTTGTA ATGGAGAAAA CCGTACAACA TTAAGTGGTG GAGGGCCATG

Figure 26 AJ

34201 GCTGGCCAAA ACCTGCCCCG CGGCTATACA CTGCAGGGAA CCGGGACTGG
 CGACCGGTTT TGGACGGGCG GCCGATATGT GACGTCCCTT GGCCCTGACC

 34251 AACAAATGACA GTGGAGAGCC CAGGACTCGT AACCATGGAT CATCATGCTC
 TTGTTACTGT CACCTCTCGG GTCCTGAGCA TTGGTACCTA GTAGTACGAG

 34301 GTCATGATAT CAATGTTGGC ACAACACAGG CACACGTGCA TACACTTCCT
 CAGTACTATA GTTACAACCG TGTGTGTGCC GTGTGCACGT ATGTGAAGGA

 34351 CAGGATTACA AGCTCCTCCC GCGTTAGAAC CATATCCCAG GGAACAACCC
 GTCCTAATGT TCGAGGAGGG CGCAATCTTG GTATAGGGTC CCTTGTGGG

 34401 ATTCCTGAAT CAGCGTAAAT CCCACACTGC AGGGAAGACC TCGCACGTAA
 TAAGGACTTA GTCGCATTTA GGGTGTGACG TCCCTTCTGG AGCGTGCATT

 34451 CTCACGTTGT GCATTGTCAA AGTGTTACAT TCGGGCAGCA GCGGATGATC
 GAGTGCAACA CGTAACAGTT TCACAATGTA AGCCCGTCGT CGCCTACTAG

 34501 CTCCAGTATG GTAGCGCGGG TTTCTGTCTC AAAAGGAGGT AGACGATCCC
 GAGGTCATAC CATCGCGCCC AAAGACAGAG TTTTCCTCCA TCTGCTAGGG

 34551 TACTGTACGG AGTGCGCCGA GACAACCGAG ATCGTGTGG TCGTAGTGTC
 ATGACATGCC TCACGCGGCT CTGTTGGCTC TAGCACAACC AGCATCACAG

 34601 ATGCCAAATG GAACGCCCGA CGTAGTCATA TTTCTGAAG CAAAACCAGG
 TACGGTTTAC CTTGCGGCCCT GCATCAGTAT AAAGGACTTC GTTTTGGTCC

 34651 TGCGGGCGTG ACAAACAGAT CTGCGTCTCC GGTCTCGCCG CTTAGATCGC
 ACGCCCGCAC TGTTTGTCTA GACGCAGAGG CCAGAGCGGC GAATCTAGCG

 34701 TCTGTGTAGT AGTTGTAGTA TATCCACTCT CTCAAAGCAT CCAGGCGCCC
 AGACACATCA TCAACATCAT ATAGGTGAGA GAGTTTCGTA GGTCCGCGGG

 34751 CCTGGCTTCG GGTTCTATGT AACTCCTTC ATGCGCCGCT GCCCTGATAA
 GGACCGAAGC CCAAGATACA TTTGAGGAAG TACGCGGCGA CGGGACTATT

 34801 CATCCACCAC CGCAGAATAA GCCACACCCA GCCAACCTAC ACATTGTTTC
 GTAGGTGGTG GCGTCTTATT CGGTGTGGGT CGGTGGATG TGTAAGCAAG

 34851 TGCGAGTCAC ACACGGGAGG AGCGGGAAGA GCTGGAAGAA CCATGTTTTT
 ACGCTCAGTG TGTGCCCTCC TCGCCCTTCT CGACCTTCTT GGTACAAAAA

 34901 TTTTTTATTC CAAAAGATTA TCCAAAACCT CAAAATGAAG ATCTATTAAG
 AAAAAATAAG GTTTTCTAAT AGGTTTGGGA GTTTTACTTC TAGATAATTC

 34951 TGAACGCGCT CCCCTCCGGT GCGTGGTCA AACTCTACAG CCAAAGAACA
 ACTTGCGCGA GGGGAGGCCA CCGCACCAGT TTGAGATGTC GGTTCCTTGT

 35001 GATAATGGCA TTTGTAAGAT GTTGACAAAT GGCTTCCAAA AGGCAAACGG
 CTATTACCGT AAACATTCTA CAACGTGTTA CCGAAGGTTT TCCGTTTGCC

 35051 CCCTCACGTC CAAGTGGACG TAAAGGCTAA ACCCTTCAGG GTGAATCTCC
 GGGAGTGCAG GTTCACCTGC ATTTCCGATT TGGGAAGTCC CACTTAGAGG

Figure 26 AK


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35151 CCACCTTCTC AATATATCTC TAAGCAAATC CCGAATATTA AGTCCGGCCA
      GGTGGAAGAG TTATATAGAG ATTCGTTTAG GGCTTATAAT TCAGGCCGGT

35201 TTGTAAAAAT CTGCTCCAGA GCGCCCTCCA CCTTCAGCCT CAAGCAGCGA
      AACATTTTFA GACGAGGTCT CGCGGGAGGT GGAAGTCGGA GTTCGTCGCT

35251 ATCATGATTG CAAAAATTCA GGTTCCTCAC AGACCTGTAT AAGATTCAAA
      TAGTACTAAC GTTTTAAAGT CCAAGGAGTG TCTGGACATA TTCTAAGTTT

35301 AGCGGAACAT TAACAAAAAT ACCGCGATCC CGTAGGTCCC TTCGCAGGGC
      TCGCCTTGTA ATTGTTTTTA TGGCGCTAGG GCATCCAGGG AAGCGTCCCG

35351 CAGCTGAACA TAATCGTGCA GGTCTGCACG GACCAGCGCG GCCACTTCCC
      GTCGACTTGT ATTAGCACGT CCAGACGTGC CTGGTCGCGC CGGTGAAGGG

35401 CGCCAGGAAC CATGACAAAA GAACCCACAC TGATTATGAC ACGCATACTC
      GCGGTCCCTG GTACTGTTTT CTTGGGTGTG ACTAATACTG TCGGTATGAG

35451 GGAGCTATGC TAACCAGCGT AGCCCCGATG TAAGCTTGTT GCATGGGCGG
      CCTCGATACG ATTGGTCGCA TCGGGGCTAC ATTCGAACAA CGTACCCGCC

35501 CGATATAAAA TGCAAGGTGC TGCTCAAAAA ATCAGGCAAA GCCTCGCGCA
      GCTATATTTT ACGTTCCACG ACGAGTTTTT TAGTCCGTTT CGGAGCGCGT

35551 AAAAAAGAAAG CACATCGTAG TCATGCTCAT GCAGATAAAG GCAGGTAAGC
      TTTTCTTTC GTGTAGCATC AGTACGAGTA CGTCTATTTC CGTCCATTTC

35601 TCCGGAACCA CCACAGAAAA AGACACCATT TTTCTCTCAA ACATGTCTGC
      AGGCCTTGTT GGTGTCTTTT TCTGTGGTAA AAAGAGAGTT TGTACAGACG

35651 GGGTTTCTGC ATAAACACAA AATAAAATAA CAAAAAACA TTTAAACATT
      CCCAAAGACG TATTTGTGTT TTATTTTATT GTTTTTTTGT AAATTTGTAA

35701 AGAAGCCTGT CTTACAACAG GAAAAACAAC CCTTATAAGC ATAAGACGGA
      TCTTCGACA GAATGTTGTC CTTTTTGTTG GGAATATTCG TATTCTGCCT

35751 CTACGGCCAT GCCGGCGTGA CCGTAAAAAA ACTGGTCACC GTGATTAAAA
      GATGCCGGTA CGGCCGCACT GGCATTTTTT TGACCAGTGG CACTAATTTT

35801 AGCACCACCG ACAGCTCCTC GGTTCATGTC GGAGTCATAA TGTAAGACTC
      TCGTGGTGGC TGTCGAGGAG CCAGTACAGG CCTCAGTATT ACATTCTGAG

35851 GGTAAACACA TCAGGTTGAT TCACATCGGT CAGTGCTAAA AAGCGACCGA
      CCATTTGTGT AGTCCAACTA AGTGTAGCCA GTCACGATTT TTCGCTGGCT

35901 AATAGCCCGG GGAATACAT ACCCGCAGGC GTAGAGACAA CATTACAGCC
      TTATCGGGCC CCCTTATGTA TGGGCGTCCG CATCTCTGTT GTAATGTCGG

35951 CCCATAGGAG GTATAACAAA ATTAATAGGA GAGAAAAACA CATAAACACC
      GGGTATCCTC CATATTGTTT TAATTATCCT CTCTTTTTGT GTATTGTGG

36001 TGAAAAACCC TCCTGCCTAG GCAAAATAGC ACCCTCCCGC TCCAGAACAA
      ACTTTTTGGG AGGACGGATC CGTTTTATCG TGGGAGGGCG AGGTCTTGTT

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Figure 26 AL

36101 AAAGAAAACC TATTAAAAAA ACACCACTCG ACACGGCACC AGCTCAATCA
 TTTCTTTTGG ATAATTTTTT TGTGGTGAGC TGTGCCGTGG TCGAGTTAGT

 36151 GTCACAGTGT AAAAAAGGGC CAAGTGCAGA GCGAGTATAT ATAGGACTAA
 CAGTGTACACA TTTTTCCTCCG GTTCACGTCT CGCTCATATA TATCCTGATT

 36201 AAAATGACGT AACGGTTAAA GTCCACAAAA AACACCCAGA AAACCGCACG
 TTTTACTGCA TTGCCAATTT CAGGTGTTTT TTGTGGGTCT TTTGGCGTGC

 36251 CGAACCTACG CCCAGAAACG AAAGCCAAAA AACCCACAAC TTCCTCAAAT
 GCTTGGATGC GGGTCTTTGC TTTTCGGTTTT TTGGGTGTTG AAGGAGTTTA

 36301 CGTCACTTCC GTTTTCCCAC GTTACGTCAC TTCCCATTTT AAGAAAATA
 GCAGTGAAGG CAAAAGGGTG CAATGCAGTG AAGGGTAAAA TTCTTTTGAT

 36351 CAATTCCCAA CACATACAAG TTAATCCGCC CTAAAACCTA CGTCACCCGC
 GTTAAGGGTT GTGTATGTTT AATGAGGCGG GATTTTGGAT GCAGTGGGCG

 36401 CCCGTTCCCA CGCCCCGCGC CACGTCACAA ACTCCACCCC CTCATTATCA
 GGGCAAGGGT GCGGGGCGCG GTGCAGTGTT TGAGGTGGGG GAGTAATAGT

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 36451 TATTGGCTTC AATCCAAAAT AAGGTATATT ATTGATGATG TTAATTAAGA
 ATAACCGAAG TTAGGTTTTA TTCCATATAA TAACTACTAC AATTAATTCT

 36501 ATTCGGATCT GCGACGCGAG GCTGGATGGC CTTCCCCATT ATGATTCTTC
 TAAGCCTAGA CGCTGCGCTC CGACCTACCG GAAGGGGTAA TACTAAGAAG

 36551 TCGCTTCCGG CGGCATCGGG ATGCCCCGCT TGCAGGCCAT GCTGTCCAGG
 AGCGAAGGCC GCCGTAGCCC TACGGGCGCA ACGTCCGGTA CGACAGGTCC

 36601 CAGGTAGATG ACGACCATCA GGGACAGCTT CAAGGCCAGC AAAAGGCCAG
 GTCCATCTAC TGCTGGTAGT CCCTGTCGAA GTTCCGGTCG TTTTCCGGTC

 36651 GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC
 CTTGGCATT TTTCCGGCGCA ACGACCGCAA AAAGGTATCC GAGGCGGGGG

 36701 CTGACGAGCA TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG
 GACTGCTCGT AGTGTTTTTA GCTGCGAGTT CAGTCTCCAC CGCTTTGGGC

 36751 ACAGGACTAT AAAGATACCA GGCCTTTCCC CCTGGAAGCT CCCTCGTGCG
 TGTCTGATA TTTCTATGGT CCGCAAAGGG GGACCTTCGA GGGAGCACGC

 36801 CTCTCCTGTT CCGACCCTGC CGCTTACCGG ATACCTGTCC GCCTTTCTCC
 GAGAGGACAA GGCTGGGACG GCGAATGGCC TATGGACAGG CGGAAAGAGG

 36851 CTTCCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG GTATCTCAGT
 GAAGCCCTTC GCACCGCGAA AGAGTATCGA GTGCGACATC CATAGAGTCA

 36901 TCGGTGTAGG TCGTTGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT
 AGCCACATCC AGCAAGCGAG GTTCGACCCG ACACACGTGC TTGGGGGGCA

Figure 26 AM

37001 CGGTAAGACA CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT
 GCCATTCTGT GCTGAATAGC GGTGACCGTC GTCGGTGACC ATTGTCTTAA

 37051 AGCAGAGCGA GGTATGTAGG CGGTGCTACA GAGTTCTTGA AGTGGTGCCC
 TCGTCTCGCT CCATACATCC GCCACGATGT CTCAAGAACT TCACCACCGG

 37101 TAACTACGGC TACACTAGAA GGACAGTATT TGGTATCTGC GCTCTGCTGA
 ATTGATGCCG ATGTGATCTT CCTGTCATAA ACCATAGACG CGAGACGACT

 37151 AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC CGGCAAAACA
 TCGGTCAATG GAAGCCTTTT TCTCAACCAT CGAGAACTAG GCCGTTTGTT

 37201 ACCACCGCTG GTAGCGGTGG TTTTMTTGTT TGCAAGCAGC AGATTACGCG
 TGGTGGCGAC CATCGCCACC AAAAAACAA ACGTTCGTCT TCTAATGCGC

 37251 CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG
 GTCTTTTTTT CCTAGAGTTC TTCTAGGAAA CTAGAAAAGA TGCCCCAGAC

 37301 ACGCTCAGTG GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA
 TGCGAGTCAC CTTGCTTTTG AGTGCAATTC CCTAAAACCA GTACTCTAAT

 37351 TCAAAAAGGA TCTTCACCTA GATCCTTTTA AATCAATCTA AAGTATATAT
 AGTTTTTCCT AGAAGTGGAT CTAGGAAAAT TTAGTTAGAT TTCATATATA

 37401 GAGTAAACTT GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT
 CTCATTTGAA CCAGACTGTC AATGGTTACG AATTAGTCAC TCCGTGGATA

 37451 CTCAGCGATC TGTCTATTTT GTTCATCCAT AGTTGCCTGA CTCCCCGTCG
 GAGTCGCTAG ACAGATAAAG CAAGTAGGTA TCAACGGACT GAGGGGCAGC

 37501 TG TAGATAAC TACGATACGG GAGGGCTTAC CATCTGGCCC CAGTGCTGCA
 ACATCTATTG ATGCTATGCC CTCCCGAATG GTAGACCGGG GTCACGACGT

 37551 ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT CAGCAATAAA
 TACTATGGCG CTCTGGGTGC GAGTGGCCGA GGTCTAAATA GTCGTTATTT

 37601 CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCTGCA ACTTTATCCG
 GGTGGTTCGG CCTTCCCGGC TCGCGTCTTC ACCAGGACGT TGAAATAGGC

 37651 CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCTG
 GGAGGTAGGT CAGATAATTA ACAACGGCCC TTCGATCTCA TTCATCAAGC

 37701 CCAGTTAATA GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT
 GGTCAATTAT CAAACGCGTT GCAACAACGG TAACGATGTC CGTAGCACCA

 37751 GTCACGCTCG TCGTTTGGTA TGGCTTCATT CAGCTCCGGT TCCCAACGAT
 CAGTGCGAGC AGCAAACCAT ACCGAAGTAA GTCGAGGCCA AGGGTTGCTA

 37801 CAAGGCGAGT TACATGATCC CCCATGTTGT GCAAAAAAGC GGTAGCTCC
 GTTCCGCTCA ATGTACTAGG GGGTACAACA CGTTTTTTCG CCAATCGAGG

 37851 TTCGGTCCTC CGATCGTTGT CAGAAGTAAG TTGGCCGCAG TGTATCACT
 AAGCCAGGAG GCTAGCAACA GTCTTCATTC AACCGGCGTC ACAATACTGA

Figure 26 A N

37951 GATGCTTTTC TGTGACTGGT GAGTACTCAA CCAAGTCATT CTGAGAATAG
 CTACGAAAAG ACACTGACCA CTCATGAGTT GGTTCAGTAA GACTCTTATC

 38001 TGTATGCGGC GACCGAGTTG CTCTTGCCCG GCGTCAACAC GGGATAATAC
 ACATACGCCG CTGGCTCAAC GAGAACGGGC CGCAGTTGTG CCCTATTATG

 38051 CGCGCCACAT AGCAGAACTT TAAAAGTGCT CATCATTTGA AAACGTTCTT
 GCGCGGTGTA TCGTCTTGAA ATTTTCACGA GTAGTAACCT TTTGCAAGAA

 38101 CGGGGCGAAA ACTCTCAAGG ATCTTACCGC TGTGAGATC CAGTTCGATG
 GCGCGCTTT TGAGAGTTCC TAGAATGGCG ACAACTCTAG GTCAAGCTAC

 38151 TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA CTTTCACCAG
 ATTGGGTGAG CACGTGGGTT GACTAGAAGT CGTAGAAAAT GAAAGTGGTC

 38201 CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA
 GCAAAGACCC ACTCGTTTTT GTCCTTCCGT TTTACGGCGT TTTTCCCTT

 38251 TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT
 ATTCCCCTG TGCCTTTACA ACTTATGAGT ATGAGAAGGA AAAAGTTATA

 38301 TATTGAAGCA TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA
 ATAACCTCGT AAATAGTCCC AATAACAGAG TACTCGCCTA TGTATAAACT

 38351 ATGTATTTAG AAAAATAAAC AAATAGGGGT TCCGCGCACA TTTCCCCGAA
 TACATAAATC TTTTATTTG TTTATCCCCA AGGCGCGTGT AAAGGGGCTT

 38401 AAGTGCCACC TGACGTCTAA GAAACCATTA TTATCATGAC ATTAACCTAT
 TTCACGGTGG ACTGCAGATT CTTTGGTAAT AATAGTACTG TAATTGGATA

 38451 AAAAATAGGC GTATCACGAG GCCCTTTCGT CTTCAAGAAT TGGATCCGAA
 TTTTATCCG CATAGTGCTC CGGGAAAGCA GAAGTTCTTA ACCTAGGCTT

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38501 TTCTTAATTT CTTAATTAA (SEQ ID NO:32)
 AAGAATTAAA GAATTAATT (SEQ ID NO:33)

Figure 26 A0

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1  CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG
   GTAGTAGTTA TTATATGGAA TAAACCTAA CTTCGGTTAT ACTATTACTC

51  GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG
   CCCCACCTCA AACACTGCAC CGCGCCCCGC ACCCTTGCCC CGCCCACTGC

101 TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA
   ATCATCACAC CGCCTTCACA CTACAACGTT CACACCGCCT TGTGTACATT

151 GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG
   CGCTGCCTAC ACCGTTTTCA CTGCAAAAAC CACACGCGGC CACATGTGTC

201 GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG
   CTTCACTGTT AAAAGCGCGC CAAATCCGC CTACAACATC ATTTAAACCC

251 CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAACTG AATAAGAGGA
   GCATTGGCTC ATTCTAAACC GGTAAAAGCG CCCTTTTGAC TTATTCTCCT

301 AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA
   TCACCTTAGA CTTATTAAAA CACAATGAGT ATCGCGCATT ATAAACAGAT

351 GGGCGCGGGG GACTTTGACC GTTTACGTGG AGACTCGCCC AGGTGTTTTT
   CCCGGCGCCC CTGAACTGG CAAATGCACC TCTGAGCGGG TCCACAAAAA

401 CTCAGGTGTT TTCCGCGTTC CGGGTCAAAG TTGGCGTTTT ATTATTATAG
   GAGTCCACAA AAGGCGCAAG GCCCAGTTTC AACCGCAAAA TAATAATATC

451 GCGGCCGCGA TCCATTGCAT ACGTTGTATC CATATCATAA TATGTACATT
   CGCCGGCGCT AGGTAACGTA TGCAACATAG GTATAGTATT ATACATGTAA

501 TATATTGGCT CATGTCCAAC ATTACCGCCA TGTTGACATT GATTATTGAC
   ATATAACCGA GTACAGTTG TAATGGCGGT ACAACTGTAA CTAATAACTG

551 TAGTTATTAA TAGTAATCAA TTACGGGGTC ATTAGTTCAT AGCCCATATA
   ATCAATAATT ATCATTAGTT AATGCCCCAG TAATCAAGTA TCGGGTATAT

601 TGGAGTTCCG CGTTACATAA CTTACGGTAA ATGGCCCGCC TGGCTGACCG
   ACCTCAAGGC GCAATGTATT GAATGCCATT TACCGGGCGG ACCGACTGGC

651 CCCAACGACC CCCGCCATT GACGTCAATA ATGACGTATG TTCCCATAGT
   GGGTTGCTGG GGGCGGGTAA CTGCAGTTAT TACTGCATAC AAGGGTATCA

701 AACGCCAATA GGGACTTTCC ATTGACGTCA ATGGGTGGAG TATTTACGGT
   TTGCGGTTAT CCCTGAAAGG TAACTGCAGT TACCCACCTC ATAAATGCCA

751 AAAGTCCCCA CTTGGCAGTA CATCAAGTGT ATCATATGCC AAGTACGCCC
   TTTGACGGGT GAACCGTCAT GTAGTTCACA TAGTATACGG TTCATGCGGG

801 CCTATTGACG TCAATGACGG TAAATGGCCC GCCTGGCATT ATGCCCAGTA
   GGATAACTGC AGTTACTGCC ATTTACGGGG CGGACCGTAA TACGGGTCAT

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Figure 27A

901 TCGCTATTAC CATGGTGATG CGGTTTTGGC AGTACATCAA TGGGCGTGGA
 AGCGATAATG GTACCACTAC GCCAAAACCG TCATGTAGTT ACCCGCACCT

 951 TAGCGGTTTG ACTCACGGGG ATTTCCAAGT CTCCACCCCA TTGACGTCAA
 ATCGCCAAAC TGAGTGCCCC TAAAGGTTCA GAGGTGGGGT AACTGCAGTT

 1001 TGGGAGTTTG TTTTGGCACC AAAATCAACG GGACTTTCCA AAATGTCGTA
 ACCCTCAAAC AAAACCGTGG TTTTAGTTGC CCTGAAAGGT TTTACAGCAT

 1051 ACAACTCCGC CCCATTGACG CAAATGGGCG GTAGGCGTGT ACGGTGGGAG
 TGTTGAGGCG GGGTAACTGC GTTTACCCGC CATCCGCACA TGCCACCCTC

 1101 GTCTATATAA GCAGAGCTCG TTTAGTGAAC CGTCAGATCG CCTGGAGACG
 CAGATATATT CGTCTCGAGC AAATCACTTG GCAGTCTAGC GGACCTCTGC

 1151 CCATCCACGC TGTTTTGACC TCCATAGAAG ACACCGGGAC CGATCCAGCC
 GGTAGGTGCG ACAAACCTGG AGGTATCTTC TGTGGCCCTG GCTAGGTGCG

 1201 TCCGCGGCCG GGAACGGTGC ATTGGAACGC GGATTCCCCG TGCCAAGAGT
 AGGCGCCGCG CCTTGCCACG TAACCTTGCG CCTAAGGGGC ACGGTTCTCA

 1251 GAGATCTGCC ACCATGGCCG GCAAGTGGTC CAAGAGGTCC GTGCCCGGCT
 CTCTAGACGG TGGTACCGGC CGTTCACCAG GTTCTCCAGG CACGGGCCGA

 1301 GGTCCACCGT GAGGGAGAGG ATGAGGAGGG CCGAGCCCGC CGCCGACAGG
 CCAGGTGGCA CTCCCTCTCC TACTCCTCCC GGCTCGGGCG GCGGCTGTCC

 1351 GTGAGGAGGA CCGAGCCCGC CGCAGTGGGC GTGGGCGCCG TGTCCAGGGA
 CACTCCTCCT GGCTCGGGCG GCGTCACCCG CACCCGCGGC ACAGGTCCCT

 1401 CCTGGAGAAG CACGGCGCCA TCACCTCCTC CAACACCGCC GCCACCAACG
 GGACCTCTTC GTGCCGCGGT AGTGGAGGAG GTTGTGGCGG CGGTGGTTGC

 1451 CCGACTGCGC CTGGCTGGAG GCCCAGGAGG ACGAGGAGGT GGGCTTCCCC
 GGCTGACGCG GACCGACCTC CGGGTCCTCC TGCTCCTCCA CCCGAAGGGG

 1501 GTGAGGCCCC AGGTGCCCCCT GAGGCCCATG ACCTACAAGG GCGCCGTGGA
 CACTCCGGGG TCCACGGGGA CTCCGGGTAC TGGATGTTCC CGCGGCACCT

 1551 CCTGTCCCAC TTCCTGAAGG AGAAGGGCGG CCTGGAGGGC CTGATCCACT
 GGACAGGGTG AAGGACTTCC TCTTCCCGCC GGACCTCCCG GACTAGGTGA

 1601 CCCAGAAGAG GCAGGACATC CTGGACCTGT GGGTGTACCA CACCCAGGGC
 GGGTCTTCTC CGTCCTGTAG GACCTGGACA CCCACATGGT GTGGGTCCCC

 1651 TACTTCCCCG ACTGGCAGAA CTACACCCCC GGCCCCGGCA TCAGGTTCCC
 ATGAAGGGGC TGACCGTCTT GATGTGGGGG CCGGGGCCGT AGTCCAAGGG

 1701 CCTGACCTTC GGCTGGTGCT TCAAGCTGGT GCCCCTGGAG CCCGAGAAGG
 GGACTGGAAG CCGACCACGA AGTTCGACCA CGGGCACCTC GGGCTCTTCC

 1751 TGGAGGAGGC CAACGAGGGC GAGAACAAC TCGCCGCCCA CCCCATGTCC
 ACCTCCTCCG GTTGCTCCCG CTCTTGTTGA CGCGGCGGGT GGGGTACAGG

Figure 27B

| | | | | | |
|------|---------------------------|--------------------------|--------------------------|---------------------------|---------------------------|
| 1851 | CTCCAAGCTG GAGGTTTCGAC | GCCTTCCACC CGGAAGGTGG | ACGTGGCCAG TGCACCGGTC | GGAGCTGCAC CCTCGACGTG | CCCAGTACT GGGCTCATGA |
| 1901 | ACAAGGACTG TGTTCCCTGAC | CTAAAGCCCG GATTTTCGGG | GGCAGATCTG CCGTCTAGAC | CTGTGCCTTC GACACGGAAG | TAGTTGCCAG ATCAACGGTC |
| 1951 | CCATCTGTTG GGTAGACAAC | TTTGCCCTC AAACGGGGAG | CCCCGTGCCT GGGGCACGGA | TCCTTGACCC AGGAACTGGG | TGGAAGGTGC ACCTTCCACG |
| 2001 | CACTCCCACT GTGAGGGTGA | GTCCTTTCCT CAGGAAAGGA | AATAAAATGA TTATTTTACT | GGAAATTGCA CCTTTAACGT | TCGCATTGTC AGCGTAACAG |
| 2051 | TGAGTAGGTG ACTCATCCAC | TCATTCTATT AGTAAGATAA | CTGGGGGGTG GACCCCCCAC | GGGTGGGGCA CCCACCCCGT | GGACAGCAAG CCTGTCGTTC |
| 2101 | GGGGAGGATT CCCCCTCTAA | GGGAAGACAA CCCTTCTGTT | TAGCAGGCAT ATCGTCCGTA | GCTGGGGATG CGACCCCTAC | CGGTGGGGCTC GCCACCCGAG |
| 2151 | TATGGCCGAT ATACCGGCTA | CGGCGCGCCG GCCGCGCGGC | TACTGAAATG ATGACTTTAC | TGTGGGCGTG ACACCCGCAC | GCTTAAGGGT CGAATTCCCA |
| 2201 | GGGAAAGAAT CCCTTTCTTA | ATATAAGGTG TATATTCCAC | GGGGTCTTAT CCCCAGAATA | GTAGTTTTGT CATCAAAACA | ATCTGTTTTG TAGACAAAAC |
| 2251 | CAGCAGCCGC GTCGTCGGCG | CGCCGCCATG GCGGCGGTAC | AGCACCAACT TCGTGGTTGA | CGTTTGATGG GCAAACCTACC | AAGCATTGTC TTCGTAACAC |
| 2301 | AGCTCATATT TCGAGTATAA | TGACAACGCG ACTGTTGCGC | CATGCCCCCA GTACGGGGGT | TGGGCCGGGG ACCCGGCCCC | TGCGTCAGAA ACGCAGTCTT |
| 2351 | TGTGATGGGC ACACTACCCG | TCCAGCATTG AGGTCGTAAC | ATGGTCGCCC TACCAGCGGG | CGTCCTGCCC GCAGGACGGG | GCAAACCTA CGTTTGAGAT |
| 2401 | CTACCTTGAC GATGGAACCTG | CTACGAGACC GATGCTCTGG | GTGTCTGGAA CACAGACCTT | CGCCGTTGGA GCGGCAACCT | GA CTGCAGCC CTGACGTCGG |
| 2451 | TCCGCCGCCG AGGCGGCGGC | CTTCAGCCGC GAAGTCGGCG | TGCAGCCACC ACGTCGGTGG | GCCCGCGGGA CGGGCGCCCT | TTGTGACTGA AACACTGACT |
| 2501 | CTTTGCTTTC GAAACGAAAG | CTGAGCCCGC GACTCGGGCG | TTGCAAACAG AACGTTTGTG | TGCAGCTTCC ACGTCGAAGG | CGTTCATCCG GCAAGTAGGC |
| 2551 | CCCGCGATGA GGGCGCTACT | CAAGTTGACG GTTCAACTGC | GCTCTTTTGG CGAGAAAACC | CACAAATTGGA GTGTTAACCT | TTCTTTGACC AAGAACTGG |
| 2601 | CGGGAACCTA GCCCTTGAAT | ATGTCGTTTC TACAGCAAAG | TCAGCAGCTG AGTCGTCGAC | TTGGATCTGC AACCTAGACG | GCCAGCAGGT CGGTCGTCCA |
| 2651 | TTCTGCCCTG AAGACGGGAC | AAGGCTTCCT TTCCGAAGGA | CCCCTCCCAA GGGGAGGGTT | TGCGGTTTAA ACGCCAAATT | AACATAAATA TTGTATTTAT |
| 2701 | AAAAACCAGA TTTTTGGTCT | CTCTGTTTGG GAGACAAACC | ATTTGGATCA TAAACCTAGT | AGCAAGTGTC TCGTTACACG | TTGCTGTCTT AACGACAGAA |

Figure 27C

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2751 TATTTAGGGG TTTTGC GCGC GCGGTAGGCC CGGGACCAGC GGTCTCGGTC
      ATAAATCCCC AAAACGCGCG CGCCATCCGG GCCCTGGTCG CCAGAGCCAG

2801 GTTGAGGGTC CTGTGTATTT TTTCCAGGAC GTGGTAAAGG TGA CTCTGGA
      CAACTCCAG GACACATAAA AAAGGTCCTG CACCATTTC ACTGAGACCT

2851 TGTTCAGATA CATGGGCATA AGCCCGTCTC TGGGGTGGAG GTAGCACCAC
      ACAAGTCTAT GTACCCGTAT TCGGGCAGAG ACCCCACCTC CATCGTGGTG

2901 TGCAGAGCTT CATGCTGCGG GGTGGTGTG TAGATGATCC AGTCGTAGCA
      ACGTCTCGAA GTACGACGCC CCACCACAAC ATCTACTAGG TCAGCATCGT

2951 GGAGCGCTGG GCGTGGTGCC TAAAAATGTC TTTCAGTAGC AAGCTGATTG
      CCTCGCGACC CGCACCACGG ATTTTACAG AAAGTCATCG TTCGACTAAC

3001 CCAGGGGCAG GCCCTTGGTG TAAGTGTTTA CAAAGCGGTT AAGCTGGGAT
      GGTCCCCGTC CGGGAACCAC ATTCACAAAT GTTTCGCCAA TTCGACCCTA

3051 GGGTGCATAC GTGGGGATAT GAGATGCATC TTGGACTGTA TTTTAGGTT
      CCCACGTATG CACCCCTATA CTCTACGTAG AACCTGACAT AAAAATCCAA

3101 GGCTATGTTT CCAGCCATAT CCCTCCGGGG ATTCATGTTG TGCAGAACCA
      CCGATAACAAG GGTCCGTATA GGGAGGCCCC TAAGTACAAC ACGTCTTGGT

3151 CCAGCACAGT GTATCCGGTG CACTTGGGAA ATTTGTCATG TAGCTTAGAA
      GGTCTGTGCA CATAGGCCAC GTGAACCCTT TAAACAGTAC ATCGAATCTT

3201 GGAAATGCGT GGAAGAACTT GGAGACGCCC TTGTGACCTC CAAGATTTTC
      CCTTTACGCA CCTTCTTGAA CCTCTGCGGG AACACTGGAG GTTCTAAAAG

3251 CATGCATTCT TCCATAATGA TGGCAATGGG CCCACGGGCG GCGGCCTGGG
      GTACGTAAGC AGGTATTACT ACCGTTACCC GGGTGCCCCG CGCCGGACCC

3301 CGAAGATATT TCTGGGATCA CTAACGTCAT AGTTGTGTTT CAGGATGAGA
      GCTTCTATAA AGACCCTAGT GATTGCAGTA TCAACACAAG GTCCTACTCT

3351 TCGTCATAGG CCATTTTAC AAAGCGCGGG CGGAGGGTGC CAGACTGCGG
      AGCAGTATCC GGTAAAAATG TTTCGCGCCC GCCTCCACG GTCTGACGCC

3401 TATAATGGTT CCATCCGGCC CAGGGGCGTA GTTACCCTCA CAGATTTGCA
      ATATTACCAA GGTAGGCCGG GTCCCCGCAT CAATGGGAGT GTCTAAACGT

3451 TTTCCACGCG TTTGAGTTCA GATGGGGGGA TCATGTCTAC CTGCGGGGCG
      AAAGGGTGCG AAACCTCAAGT CTACCCCCCT AGTACAGATG GACGCCCCGCG

3501 ATGAAGAAAA CGGTTTCCGG GGTAGGGGAG ATCAGCTGGG AAGAAAGCAG
      TACTTCTTTT GCCAAAGGCC CCATCCCCCT TAGTCGACCC TTCTTTTCGT

3551 GTTCCTGAGC AGCTGCGACT TACCGCAGCC GGTGGGCCCC TAAATCACAC
      CAAGGACTCG TCGACGCTGA ATGGCGTCGG CCACCCGGGC ATTTAGTGTG

3601 CTATTACCGG CTGCAACTGG TAGTTAAGAG AGCTGCAGCT GCCGTCTATC
      GATAATGGCC GACGTTGACC ATCAATTCTC TCGACGTGCA CGGCAGTAGG

3651 CTGAGCAGGG GGGCCACTTC GTTAAGCATG TCCCTGACTC GCATGTTTTT
      GACTCGTCCC CCCGGTGAAG CAATTCGTAC AGGGACTGAG CGTACAAAAG

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Figure 27 D

3701 CCTGACCAAA TCCGCCAGAA GGCCTCGCC GCCAGCGAT AGCAGTTCTT
GGACTGGTTT AGGCGGTCTT CCGCGAGCGG CGGGTCGCTA TCGTCAAGAA

3751 GCAAGGAAGC AAAGTTTTTC AACGGTTTGA GACCGTCCGC CGTAGGCATG
CGTTCCTTCG TTTCAAAAAG TTGCCAAACT CTGGCAGGCG GCATCCGTAC

3801 CTTTTGAGCG TTTGACCAAG CAGTTCCAGG CGGTCCCACA GCTCGGTCAC
GAAAACTCGC AAACCTGGTTT GTCAAGGTCC GCCAGGGTGT CGAGCCAGTG

3851 CTGCTCTACG GCATCTCGAT CCAGCATATC TCCTCGTTTC GCGGGTTGGG
GACGAGATGC CGTAGAGCTA GGTCTGTATAG AGGAGCAAAG CGCCCAACCC

3901 GCGGCTTTTCG CTGTACGGCA GTAGTCGGTG CTCGTCCAGA CGGGCCAGGG
CGCCGAAAGC GACATGCCGT CATCAGCCAC GAGCAGGTCT GCCCGGTCCC

3951 TCATGTCTTT CCACGGGCGC AGGGTCCTCG TCAGCGTAGT CTGGGTCACG
AGTACAGAAA GGTGCCCCGC TCCCAGGAGC AGTCGCATCA GACCCAGTGC

4001 GTGAAGGGGT GCGCTCCGGG CTGCGCGCTG GCCAGGGTGC GCTTGAGGCT
CACTTCCCCA CGCGAGGCCG GACGCGCGAC CGGTCCCACG CGAACTCCGA

4051 GGTCTCTGCTG GTGCTGAAGC GCTGCCGGTC TTCGCCCTGC GCGTCGGCCA
CCAGGACGAC CACGACTTCG CGACGGCCAG AAGCGGGACG CGCAGCCGGT

4101 GGTAGCATTT GACCATGGTG TCATAGTCCA GCGCTCCGC GCGGTGGCCC
CCATCGTAAA CTGGTACCAC AGTATCAGGT CGGGGAGGCG CCGCACCGGG

4151 TTGGCGCGCA GCTTGCCCTT GGAGGAGGCG CCGCACGAGG GGCAGTGCAG
AACCGCGCGT CGAACGGGAA CCTCCTCCGC GCGGTGCTCC CCGTCACGTC

4201 ACTTTTGAGG GCGTAGAGCT TGGGCGCGAG AAATACCGAT TCCGGGGAGT
TGAAAACCTC CGCATCTCGA ACCCGCGCTC TTTATGGCTA AGGCCCTCA

4251 AGGCATCCGC GCCGCAGGCC CCGCAGACGG TCTCGCATTC CACGAGCCAG
TCCGTAGGCG CGGCGTCCGG GCGGTCTGCC AGAGCGTAAG GTGCTCGGTC

4301 GTGAGCTCTG GCCGTTCCGG GTCAAAAACC AGGTTTCCCC CATGCTTTTT
CACTCGAGAC CGGCAAGCCC CAGTTTTTGG TCCAAAGGGG GTACGAAAAA

4351 GATGCGTTTC TTACCTCTGG TTTCCATGAG CCGGTGTCCA CGCTCGGTGA
CTACGCAAAG AATGGAGACC AAAGGTACTC GGCCACAGGT GCGAGCCACT

4401 CGAAAAGGCT GTCCGTGTCC CCGTATACAG ACTTGAGAGG CCTGTCTTCG
GCTTTTCCGA CAGGCACAGG GGCATATGTC TGAACCTCC GGACAGGAGC

4451 AGCGGTGTTT CGCGGTCTCT CTCGTATAGA AACTCGGACC ACTCTGAGAC
TCGCCACAAG GCGCCAGGAG GAGCATATCT TTGAGCCTGG TGAGACTCTG

4501 AAAGGCTCGC GTCCAGGCCA GCACGAAGGA GGCTAAGTGG GAGGGGTAGC
TTTCCGAGCG CAGGTCCGGT CGTGCTTCCT CCGATTACAC CTCCCCATCG

4551 GGTGCTTGTG CACTAGGGGG TCCACTCGCT CCAGGGTGTG AAGACACATG
CCAGCAACAG GTGATCCCCC AGGTGAGCGA GGTCCACAC TTCTGTGTAC

4601 TCGCCCTCTT CGGCATCAAG GAAGGTGATT GGTGTGTAGG TGTAGGCCAC
AGCGGGAGAA GCCGTAGTTC CTTCCTACTA CCAACATCC ACATCCGGTG

Figure 27E

4701 CGTCCTCACT CTCTTCCGCA TCGCTGTCTG CGAGGGCCAG CTGTTGGGGT
GCAGGAGTGA GAGAAGGCGT AGCGACAGAC GCTCCCGGTC GACAACCCCA

4751 GAGTACTCCC TCTGAAAAGC GGGCATGACT TCTGCGCTAA GATTGTCACT
CTCATGAGGG AGACTTTTTCG CCCGTACTGA AGACGCGATT CTAACAGTCA

4801 TTCCAAAAAC GAGGAGGATT TGATATTCAC CTGGCCCGCG GTGATGCCTT
AAGGTTTTTG CTCTCCTAA ACTATAAGTG GACCGGGCGC CACTACGGAA

4851 TGAGGGTGGC CGCATCCATC TGGTCAGAAA AGACAATCTT TTTGTTGTCA
ACTCCACCG GCGTAGGTAG ACCAGTCTTT TCTGTTAGAA AAACAACAGT

4901 AGCTTGGTGG CAAACGACCC GTAGAGGGCG TTGGACAGCA ACTTGCGCAT
TCGAACCACC GTTTGCTGGG CATCTCCCGC AACCTGTCTG TGAACCGCTA

4951 GGAGCGCAGG GTTTGGTTTT TGTCGCGATC GGCGCGCTCC TTGGCCGCGA
CCTCGCGTCC CAAACCAAAA ACAGCGCTAG CCGCGCGAGG AACC GGCGCT

5001 TGTTTTAGCTG CACGTATTCG CGCGCAACGC ACCGCCATTC GGGAAAGACG
ACAAATCGAC GTGCATAAGC GCGCGTTGCG TGGCGGTAAG CCCTTTCTGC

5051 GTGGTGCGCT CGTCGGGCAC CAGGTGCACG CGCCAACCGC GGTGTGTCAG
CACCACGCGA GCAGCCCGTG GTCCACGTGC GCGGTTGGCG CCAACACGTC

5101 GGTGACAAGG TCAACGCTGG TGGCTACCTC TCCGCGTAGG CGCTCGTTGG
CCACTGTTCC AGTTGCGACC ACCGATGGAG AGGCGCATCC GCGAGCAACC

5151 TCCAGCAGAG GCGGCCGCCC TTGCGCGAGC AGAATGGCGG TAGGGGGTCT
AGGTCGTCTC CGCCGGCGGG AACGCGCTCG TCTTACCGCC ATCCCCCAGA

5201 AGCTGCGTCT CGTCCGGGGG GTCTGCGTCC ACGGTAAAGA CCCC GGCGCAG
TCGACGCAGA GCAGGCCCCC CAGACGCAGG TGCCATTCTT GGGGCCGCTC

5251 CAGGCGCGCG TCGAAGTAGT CTATCTTGCA TCCTTGCAAG TCTAGCGCCT
GTCCGCGCGC AGCTTCATCA GATAGAACGT AGGAACGTT AGATCGCGGA

5301 GCTGCCATGC GCGGGCGGCA AGCGCGCGCT CGTATGGGTT GAGTGGGGGA
CGACGGTACG CGCCCGCCGT TCGCGCGCGA GCATACCCAA CTCACCCCTT

5351 CCCCATGGCA TGGGGTGGGT GAGCGCGGAG GCGTACATGC CGCAAATGTC
GGGGTACCGT ACCCCACCCA CTCGCGCCTC CGCATGTACG GCGTTTACAG

5401 GTAAACGTAG AGGGGCTCTC TGAGTATTCC AAGATATGTA GGGTAGCATC
CATTTGCATC TCCCCGAGAG ACTCATAAGG TTCTATACAT CCCATCGTAG

5451 TTCCACCGCG GATGCTGGCG CGCACGTAAT CGTATAGTTC GTGCGAGGGA
AAGGTGGCGC CTACGACCGC GCGTGATTA GCATATCAAG CACGCTCCCT

5501 GCGAGGAGGT CGGGACCGAG GTTGCTACGG GCGGGCTGCT CTGCTCGGAA
CGCTCCTCCA GCCCTGGCTC CAACGATGCC CGCCCGACGA GACGAGCCTT

5551 GACTATCTGC CTGAAGATGG CATGTGAGTT GGATGATATG GTTGGACGCT
CTGATAGACG GACTTCTACC GTACACTCAA CCTACTATAC CAACCTGCGA

Figure 27F

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5651  GAGGCGTAGG AGTCGCGCAG CTTGTTGACC AGCTCGGCGG TGACCTGCAC
      CTCCGCATCC TCAGCGCGTC GAACAACTGG TCGAGCCGCC ACTGGACGTG

5701  GTCTAGGGCG CAGTAGTCCA GGGTTTCCTT GATGATGTCA TACTTATCCT
      CAGATCCCGC GTCATCAGGT CCCAAAGGAA CTACTACAGT ATGAATAGGA

5751  GTCCCTTTTT TTTCCACAGC TCGCGGTTGA GGACAACTC TTCGCGGTCT
      CAGGGAAAAA AAAGGTGTCG AGCGCCAACT CCTGTTTGAG AAGCGCCAGA

5801  TTCCAGTACT CTTGGATCGG AAACCCGTCG GCCTCCGAAC GGTAAGAGCC
      AAGGTCATGA GAACCTAGCC TTTGGGCAGC CGGAGGCTTG CCATTCTCGG

5851  TAGCATGTAG AACTGGTTGA CGGCCTGGTA GGCGCAGCAT CCCTTTTCTA
      ATCGTACATC TTGACCAACT GCCGGACCAT CCGCGTCGTA GGGAAAAGAT

5901  CGGGTAGCGC GTATGCCTGC GCGGCCTTCC GGAGCGAGGT GTGGGTGAGC
      GCCCATCGCG CATACGGACG CGCCGGAAGG CCTCGCTCCA CACCCACTCG

5951  GCAAAGGTGT CCCTGACCAT GACTTTGAGG TACTGGTATT TGAAGTCAGT
      CGTTTCCACA GGGACTGGTA CTGAAACTCC ATGACCATAA ACTTCAGTCA

6001  GTCGTCGCAT CCGCCCTGCT CCCAGAGCAA AAAGTCCGTG CGCTTTTGTG
      CAGCAGCGTA GCGGGGACGA GGGTCTCGTT TTTCAGGCAC GCGAAAAACC

6051  AACGCGGATT TGGCAGGGCG AAGGTGACAT CGTTGAAGAG TATCTTTCCC
      TTGCGCCTAA ACCGTCCC GC TTCCACTGTA GCAACTTCTC ATAGAAAGGG

6101  GCGCGAGGCA TAAAGTTGCG TGTGATGCGG AAGGGTCCCC GCACCTCGGA
      CGCGCTCCGT ATTTCAACGC ACACTACGCC TTCCCAGGGC CGTGGAGCCT

6151  ACGGTTGTTA ATTACCTGGG CGGCGAGCAC GATCTCGTCA AAGCCGTGTA
      TGCCAACAAT TAATGGACCC GCCGCTCGTG CTAGAGCAGT TTCGGCAACT

6201  TGTTGTGGCC CACAATGTAA AGTTCCAAGA AGCGCGGGAT GCCCTTGATG
      ACAACACCGG GTGTTACATT TCAAGGTTCT TCGCGCCCTA CGGGAACCTAC

6251  GAAGGCAATT TTTTAAGTTC CTCGTAGGTG AGCTCTTCAG GGGAGCTGAG
      CTTLCGTTAA AAAATTCAAG GAGCATCCAC TCGAGAAGTC CCCTCGACTC

6301  CCCGTGCTCT GAAAGGGGCC AGTCTGCAAG ATGAGGGTTG GAAGCGACGA
      GGGCACGAGA CTTTCCCGGG TCAGACGTTT TACTCCCAAC CTTGCTGCTG

6351  ATGAGCTCCA CAGGTCACGG GCCATTAGCA TTTGCAGGTG GTCGCGAAAG
      TACTCGAGGT GTCCAGTGCC CGGTAATCGT AAACGTCCAC CAGCGCTTTC

6401  GTCCTAAACT GGCGACCTAT GGCCATTTTT TCTGGGGTGA TGCAGTAGAA
      CAGGATTTGA CCGCTGGATA CCGGTAAAAA AGACCCCACT ACGTCATCTT

6451  GGTAAGCGGG TCTTGTTCCC AGCGGTCCCA TCCAAGGTTT GCGGCTAGGT
      CCATTGCCCC AGAACAAGGG TCGCCAGGGT AGGTTCCAAG CGCCGATCCA

6501  CTCGCGCGGC AGTCACTAGA GGCTCATCTC CGCCGAACCT CATGACCAGC
      GAGCGCGCCG TCAGTGATCT CCGAGTAGAG GCGGCTTGAA GTACTGGTGC

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Figure 27G

| | | | | | |
|------|-------------|------------|-------------|------------|-------------|
| 6601 | TACATCGTAG | GTGACAAAGA | GACGCTCGGT | GCGAGGATGC | GAGCCGATCG |
| | ATGTAGCATC | CACTGTTTCT | CTGCGAGCCA | CGCTCCTACG | CTCGGCTAGC |
| 6651 | GGAAGAACTG | GATCTCCCGC | CACCAATTGG | AGGAGTGGCT | ATTGATGTGG |
| | CCTTCTTGAC | CTAGAGGGCG | GTGGTTAACC | TCCTCACCGA | TAACTACACC |
| 6701 | TGAAAGTAGA | AGTCCCTGCG | ACGGGCCGAA | CACTCGTGCT | GGCTTTTGTA |
| | ACTTTCATCT | TCAGGGACGC | TGCCCCGGCTT | GTGAGCACGA | CCGAAAACAT |
| 6751 | AAAACGTGCG | CAGTACTGGC | AGCGGTGCAC | GGGCTGTACA | TCCTGCACGA |
| | TTTTGCACGC | GTCATGACCG | TCGCCACGTG | CCCGACATGT | AGGACGTGCT |
| 6801 | GGTTGACCTG | ACGACCGCGC | ACAAGGAAGC | AGAGTGGGAA | TTTGAGCCCC |
| | CCAACTGGAC | TGCTGGCGCG | TGTTCTTTCG | TCTCACCTT | AAACTCGGGG |
| 6851 | TCGCCTGGCG | GGTTTGGCTG | GTGGTCTTCT | ACTTCGGCTG | CTTGTCCTTG |
| | AGCGGACCGC | CCAAACCGAC | CACCAGAAGA | TGAAGCCGAC | GAACAGGAAC |
| 6901 | ACCGTCTGGC | TGCTCGAGGG | GAGTTACGGT | GGATCGGACC | ACCACGCCGC |
| | TGGCAGACCG | ACGAGCTCCC | CTCAATGCCA | CCTAGCCTCG | TGGTGCGGCG |
| 6951 | GCGAGCCCAA | AGTCCAGATG | TCCGCGCGCG | GCGGTCCGAG | CTTGATGACA |
| | CGCTCGGGTT | TCAGGTCTAC | AGGCGCGCGC | CGCCAGCCTC | GAACTACTGT |
| 7001 | ACATCGCGCA | GATGGGAGCT | GTCCATGGTC | TGGAGCTCCC | GCGGCGTCAG |
| | TGTAGCGCGT | CTACCCTCGA | CAGGTACCAG | ACCTCGAGGG | CGCCGCAGTC |
| 7051 | GTCAGGCGGG | AGCTCCTGCA | GGTTTACCTC | GCATAGACGG | GTCAGGGCGC |
| | CAGTCCGCCC | TCGAGGACGT | CCAAATGGAG | CGTATCTGCC | CAGTCCC GCG |
| 7101 | GGGCTAGATC | CAGGTGATAC | CTAATTTCCA | GGGGCTGGTT | GGTGGCGGGC |
| | CCC GATCTAG | GTCCACTATG | GATTAAAGGT | CCCCGACCAA | CCACCGCCGC |
| 7151 | TCGATGGCTT | GCAAGAGGCC | GCATCCCCGC | GGCGCGACTA | CGGTACCGCG |
| | AGCTACCGAA | CGTTCTCCGG | CGTAGGGGCG | CCGCGCTGAT | GCCATGGCGC |
| 7201 | CGGCGGGCGG | TGGGCCGCGG | GGGTGTCCTT | GGATGATGCA | TCTAAAAGCG |
| | GCCGCCCCGC | ACCCGGCGCC | CCCACAGGAA | CCTACTACGT | AGATTTTCGC |
| 7251 | GTGACGCGGG | CGAGCCCCCG | GAGGTAGGGG | GGGCTCCGGA | CCCGCCGGGA |
| | CACTGCGCCC | GCTCGGGGGC | CTCCATCCCC | CCCGAGGCCT | GGGCGGCCCT |
| 7301 | GAGGGGGCAG | GGGCACGTCG | GCGCCGCGCG | CGGGCAGGAG | CTGGTGCTGC |
| | CTCCCCCGTC | CCCGTGACAG | CGCGGCGCGC | GCCCCGTCTC | GACCACGACG |
| 7351 | GCGCGTAGGT | TGCTGGCGAA | CGCGACGACG | CGGCGGTTGA | TCTCCTGAAT |
| | CGCGCATCCA | ACGACCGCTT | GCGCTGCTGC | GCCGCCAACT | AGAGGACTTA |
| 7401 | CTGGCGCCTC | TGCGTGAAGA | CGACGGGCCC | GGTGAGCTTG | AACCTGAAAG |
| | GACCGCGGAG | ACGCACTTCT | GCTGCCCGGG | CCACTCGAAC | TTGGACTTTC |
| 7451 | AGAGTTCGAC | AGAATCAATT | TCGGTGTCGT | TGACGGCGGC | CTGGCGCAAA |
| | TCTCAAGCTG | TCTTAGTTAA | AGCCACAGCA | ACTGCCGCGG | GACCGCGTTT |

Figure 27H

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7551 CTGCTCGATC TCTTCCTCCT GGAGATCTCC GCGTCCGGCT CGCTCCACGG
      GACGAGCTAG AGAAGGAGGA CCTCTAGAGG CGCAGGCCGA GCGAGGTGCC

7601 TGGCGGCGAG GTCGTTGGAA ATGCGGGCCA TGAGCTGCGA GAAGGCGTTG
      ACCGCCGCTC CAGCAACCTT TACGCCCGGT ACTCGACGCT CTTCCGCAAC

7651 AGGCCTCCCT CGTTCCAGAC GCGGCTGTAG ACCACGCCCC CTTCCGCATC
      TCCGGAGGGA GCAAGGTCTG CGCCGACATC TGGTGCGGGG GAAGCCGTAG

7701 GCGGGCGCGC ATGACCACCT GCGCGAGATT GAGCTCCACG TGCCGGGCGA
      CGCCCGCGCG TACTGGTGGA CGCGCTCTAA CTCGAGGTGC ACGGCCCGCT

7751 AGACGGCGTA GTTTCGCAGG CGCTGAAAGA GGTAGTTGAG GGTGGTGCGG
      TCTGCCGCAT CAAAGCGTCC GCGACTTTCT CCATCAACTC CCACCACCGC

7801 GTGTGTTCTG CCACGAAGAA GTACATAACC CAGCGTCGCA ACGTGGATTC
      CACACAAGAC GGTGCTTCTT CATGTATTGG GTCGCAGCGT TGCACCTAAG

7851 GTTGATATCC CCCAAGGCCT CAAGGCGCTC CATGGCCTCG TAGAAGTCCA
      CAACTATAGG GGGTTCCGGA GTTCCGCGAG GTACCGGAGC ATCTTCAGGT

7901 CGGCGAAGTT GAAAAACTGG GAGTTGCGCG CCGACACGGT TAACTCCTCC
      GCCGCTTCAA CTTTTTGACC CTCAACGCGC GGCTGTGCCA ATTGAGGAGG

7951 TCCAGAAGAC GGATGAGCTC GGCGACAGTG TCGCGCACCT CGCGCTCAAA
      AGGTCTTCTG CCTACTCGAG CCGCTGTCAC AGCGCGTGGA GCGCGAGTTT

8001 GGCTACAGGG GCCTCTTCTT CTTCTTCAAT CTCCTCTTCC ATAAGGGCCT
      CCGATGTCCC CGGAGAAGAA GAAGAAGTTA GAGGAGAAGG TATTCCCGGA

8051 CCCCTTCTTC TTCTTCTGGC GCGGTTGGGG GAGGGGGGAC ACGGCGGCGA
      GGGGAAGAAG AAGAAGACCG CCGCCACCCC CTCCCCCTG TGCCGCCGCT

8101 CGACGGCGCA CCGGGAGGCG GTCGACAAAG CGCTCGATCA TCTCCCCGCG
      GCTGCCGCGT GGCCCTCCGC CAGCTGTTTC GCGAGCTAGT AGAGGGGCGC

8151 GCGACGGCGC ATGGTCTCGG TGACGGCGCG GCCGTTCTCG CGGGGGCGCA
      CGCTGCCGCG TACCAGAGCC ACTGCCGCGC CGGCAAGAGC GCCCCCGCGT

8201 GTTGGAAGAC GCCGCCCCGTC ATGTCCCGGT TATGGGTTGG CGGGGGGCTG
      CAACCTTCTG CGGCGGGCAG TACAGGGCCA ATACCCAACC GCCCCCGCAG

8251 CCATGCGGCA GGGATACGGC GCTAACGATG CATCTCAACA ATTGTTGTGT
      GGTACGCCGT CCCTATGCCG CGATTGCTAC GTAGAGTTGT TAACAACACA

8301 AGGTACTCCG CCGCCGAGGG ACCTGAGCGA GTCCGCATCG ACCGGATCGG
      TCCATGAGGC GCGGCTCCC TGGACTCGCT CAGGCGTAGC TGGCCTAGCC

8351 AAAACCTCTC GAGAAAGGCG TCTAACCAGT CACAGTCGCA AGGTAGGCTG
      TTTTGGAGAG CTCTTTCCGC AGATTGGTCA GTGTCAGCGT TCCATCCGAC

8401 AGCACCGTGG CGGGCGGCAG CGGGCGGCGG TCGGGGTTGT TTCTGGCGGA
      TCGTGGCACC GCCCCCGGTC GCCCGCCGCC AGCCCCAACA AAGACCGCCT

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Figure 27I

| | | | | | |
|------|---------------------------|---------------------------|---------------------------|--------------------------|---------------------------|
| 8501 | TCGACAGAAAG AGCTGTCTTC | CACCATGTCC GTGGTACAGG | TTGGGTCCGG AACCCAGGCC | CCTGCTGAAT GGACGACTTA | GCGCAGGCGG CGCGTCCGCC |
| 8551 | TCGGCCATGC AGCCGGTACG | CCCAGGCTTC GGGTCCGAAG | GTTTGTGACAT CAAAACTGTA | CGGCGCAGGT GCCGCGTCCA | CTTTGTAGTA GAAACATCAT |
| 8601 | GTCTTGCAATG CAGAACGTAC | AGCCTTTCTA TCGGAAAGAT | CCGGCACTTC GGCCGTGAAG | TTCTTCTCCT AAGAAGAGGA | TCCTCTTGTC AGGAGAACAG |
| 8651 | CTGCATCTCT GACGTAGAGA | TGCATCTATC ACGTAGATAG | GCTGCGGCGG CGACGCCGCC | CGGCGGAGTT GCCGCCTCAA | TGGCCGTAGG ACCGGCATCC |
| 8701 | TGGCGCCCTC ACCGCGGGAG | TTCTTCCCAT AAGGAGGGTA | GCGTGTGACC CGCACACTGG | CCGAAGCCCC GGCTTCGGGG | TCATCGGCTG AGTAGCCGAC |
| 8751 | AAGCAGGGCT TTCGTCCCGA | AGGTCGGCGA TCCAGCCGCT | CAACGCGCTC GTTGCGCGAG | GGCTAATATG CCGATTATAC | GCCTGCTGCA CGGACGACGT |
| 8801 | CCTGCGTGAG GGACGCACTC | GGTAGACTGG CCATCTGACC | AAGTCATCCA TTCAGTAGGT | TGTCCACAAA ACAGGTGTTT | GCGGTGGTAT CGCCACCATA |
| 8851 | GCGCCCCGTGT CGCGGGCACA | TGATGGTGTA ACTACCACAT | AGTGCAGTTG TCACGTCAAC | GCCATAACGG CGGTATTGCC | ACCAGTTAAC TGGTCAATTG |
| 8901 | GSTCTGGTGA CCAGACCACT | CCCGGCTGCG GGGCCGACGC | AGAGCTCGGT TCTCGAGCCA | GTACCTGAGA CATGGACTCT | CGCGAGTAAG GCGCTCATTC |
| 8951 | CCCTCGAGTC GGGAGCTCAG | AAATACGTAG TTTATGCATC | TCGTTGCAAG AGCAACGTTT | TCCGCACCAG AGGCGTGGTC | GTA CTGGTAT CATGACCATA |
| 9001 | CCCACCAAAA GGGTGGTTTT | AGTGCGGCGG TCACGCCGCC | CGGCTGGCGG GCCGACCGCC | TAGAGGGGCC ATCTCCCCGG | AGCGTAGGGT TCGCATCCCA |
| 9051 | GGCCGGGGCT CCGGCCCCGA | CCGGGGGGCGA GGCCCCCGCT | GATCTTCCAA CTAGAAGGTT | CATAAGGCGA GTATTCCGCT | TGATATCCGT ACTATAGGCA |
| 9101 | AGATGTACCT TCTACATGGA | GGACATCCAG CCTGTAGGTC | GTGATGCCGG CACTACGGCC | CGGCGGTGGT GCCGCCACCA | GGAGGCGCGC CCTCCGCGCG |
| 9151 | GGAAAGTCGC CCTTTCAGCG | GGACGCGGTT CCTGCGCCAA | CCAGATGTTG GGTCTACAAC | CGCAGCGGCA GCGTCGCCGT | AAAAGTGCTC TTTTCACGAG |
| 9201 | CATGGTCGGG GTACCAGCCC | ACGCTCTGGC TGCGAGACCG | CGGTCAGGCG GCCAGTCCGC | CGCGCAATCG GCGCGTTAGC | TTGACGCTCT AACTGCGAGA |
| 9251 | AGACCGTGCA TCTGGCACGT | AAAGGAGAGC TTTCTCTCTG | CTGTAAGCGG GACATTGCGC | GCACTCTTCC CGTGAGAAGG | GTGGTCTGGT CACCAGACCA |
| 9301 | GGATAAATTC CCTATTTAAG | GCAAGGGTAT CGTTCCCAT | CATGGCGGAC GTACCGCCTG | GACCGGGGTT CTGGCCCCAA | CGAGCCCCGT GCTCGGGGCA |
| 9351 | ATCCGGCCGT TAGGCCGGCA | CCGCCGTGAT GGCGGCACTA | CCATGCGGTT GGTACGCCAA | ACCGCCCGCG TGGCGGGCGC | TGTCGAACCC ACAGCTTGGG |

Figure 27J

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9451  GGC GCG GCG CG CTGCTGCGCT AGCTTTTTTTG GCCACTGGCC GCGCGCAGCG
      CCGCGCCGCGC GACGACGCGA TCGAAAAAAC CGGTGACCGG CGCGCGTCGC

9501  TAAGCGGTTA GGCTGGAAAG CGAAAGCATT AAGTGGCTCG CTCCCTGTAG
      ATTCGCCAAT CCGACCTTTC GCTTTCGTAA TTCACCGAGC GAGGGACATC

9551  CCGGAGGGTT ATTTTCCAAG GGTGAGTCG CGGGACCCCC GGTTCGAGTC
      GGCCTCCCAA TAAAAGGTTT CCAACTCAGC GCCCTGGGGG CCAAGCTCAG

9601  TCGGACCGGC CGGACTGCGG CGAACGGGGG TTTGCCCTCCC CGTCATGCAA
      AGCCTGGCCG GCCTGACGCC GCTTGCCCCC AAACGGAGGG GCAGTACGTT

9651  GACCCCGCTT GCAAATTCCT CCGGAAACAG GGACGAGCCC CTTTTTTGCT
      CTGGGGCGAA CGTTTAAGGA GGCCTTTGTC CCTGCTCGGG GAAAAACGA

9701  TTTCCAGAT GCATCCGGTG CTGCGGCAGA TGCGCCCCC TCCTCAGCAG
      AAAGGGTCTA CGTAGGCCAC GACGCCGTCT ACGCGGGGGG AGGAGTCGTC

9751  CGGCAAGAGC AAGAGCAGCG GCAGACATGC AGGGCACCTT CCCCTCCTCC
      GCCGTTCTCG TTCTCGTCGC CGTCTGTACG TCCCGTGGGA GGGGAGGAGG

9801  TACCGCGTCA GGAGGGGCGA CATCCGCGGT TGACGCGGCA GCAGATGGTG
      ATGGCGCAGT CCTCCCCGCT GTAGGCGCCA ACTGCGCCGT CGTCTACCAC

9851  ATTACGAACC CCCGCGGCGC CGGGCCCCGGC ACTACCTGGA CTTGGAGGAG
      TAATGCTTGG GGGCGCCGCG GCCCGGGCCG TGATGGACCT GAACCTCCTC

9901  GCGGAGGGCC TGGCGCGGCT AGGAGCGCCC TCTCCTGAGC GGCACCCAAG
      CCGCTCCCGG ACCGCGCCGA TCCTCGCGGG AGAGGACTCG CCGTGGGTTC

9951  GGTGCAGCTG AAGCGTGATA CGCGTGAGGC GTACGTGCCG CGGCAGAACC
      CCACGTGCAC TTCGCACTAT GCGCACTCCG CATGCACGGC GCCGTCTTGG

10001 TGTTTTCGCA CCGCGAGGGA GAGGAGCCCC AGGAGATGCG GGATCGAAAG
      ACAAGCGCT GGCCTCCCT CTCCTCGGGC TCCTCTACGC CCTAGCTTTC

10051 TTCCACGCAG GCGCGAGCT GCGGCATGGC CTGAATCGCG AGCGGTGTGCT
      AAGGTGCGTC CCGCGCTCGA CGCCGTACCG GACTTAGCGC TCGCCAACGA

10101 GCGCGAGGAG GACTTTGAGC CCGACGCGCG AACCGGGATT AGTCCCGCGC
      CGCGCTCCTC CTGAAACTCG GGCTGCGCGC TTGGCCCTAA TCAGGGCGCG

10151 GCGCACACGT GCGGCGCCGC GACCTGGTAA CCGCATACGA GCAGACGGTG
      CGCGTGTGCA CCGCCGGCGG CTGGACCATT GCGGTATGCT CGTCTGCCAC

10201 AACCAGGAGA TTAACCTTCA AAAAAGCTTT AACAAACCAG TCGGTACGCT
      TTGGTCTCTT AATTGAAAGT TTTTTCGAAA TTGTTGGTGC ACGCATGCGA

10251 TGTGGCGCGC GAGGAGGTGG CTATAGGACT GATGCATCTG TGGGACTTTG
      ACACCGCGCG CTCCTCCACC GATATCCTGA CTACGTAGAC ACCCTGAAAC

10301 TAAGCGCGCT GGAGCAAAAC CCAAATAGCA AGCCGCTCAT GGCGCAGCTG
      ATTCGCGCGA CCTCGTTTTG GGTTTATCGT TCGGCGAGTA CCGCGTCGAC

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Figure 27K

10401 GCTAAACATA GTAGAGCCCCG AGGGCCGCTG GCTGCTCGAT TTGATAAACA
 CGATTTGTAT CATCTCGGGC TCCCGGCGAC CGACGAGCTA AACTATTTGT
 10451 TCCTGCAGAG CATAGTGGTG CAGGAGCGCA GCTTGAGCCT GGCTGACAAG
 AGGACGTCTC GTATCACCAC GTCCTCGCGT CGAACTCGGA CCGACTGTTC
 10501 GTGGCCGCCA TCAACTATTC CATGCTTAGC CTGGGCAAGT TTTACGCCCCG
 CACCGGCGGT AGTTGATAAG GTACGAATCG GACCCGTTC AATGCGGGC
 10551 CAAGATATAC CATACCCCCTT ACGTTCCCAT AGACAAGGAG GTAAAGATCG
 GTTCTATATG GTATGGGGAA TGCAAGGTA TCTGTTCCCTC CATTTCTAGC
 10601 AGGGGTTCTA CATGCGCATG GCGCTGAAGG TGCTTACCTT GAGCGACGAC
 TCCCCAAGAT GTACGCGTAC CGCGACTTCC ACGAATGGAA CTCGCTGCTG
 10651 CTGGGCGTTT ATCGCAACGA GCGCATCCAC AAGGCCGTGA GCGTGAGCCG
 GACCCGCAA TAGCGTTGCT CGCGTAGGTG TTCCGGCACT CGCACTCGGC
 10701 GCGGCGCGAG CTCAGCGACC GCGAGCTGAT GCACAGCCTG CAAAGGGCCC
 CGCCGCGCTC GAGTCGCTGG CGCTCGACTA CGTGTCGGAC GTTTCCCGGG
 10751 TGGCTGGCAC GGGCAGCGGC GATAGAGAGG CCGAGTCCTA CTTTGACGCG
 ACCGACCGTG CCCGTCGCCG CTATCTCTCC GGCTCAGGAT GAAACTGCGC
 10801 GCGGCTGACC TGCGCTGGGC CCCAAGCCGA CGCGCCCTGG AGGCAGCTGG
 CCGCGACTGG ACGCGACCCG GGGTTCGGCT GCGCGGGACC TCCGTCGACC
 10851 GGCCGGACCT GGGCTGGCGG TGGCACCCGC GCGCGCTGGC AACGTCGGCG
 CCGGCCTGGA CCCGACCGCC ACCGTGGGCG CGCGCGACCG TTGCAGCCGC
 10901 GCGTGGAGGA ATATGACGAG GACGATGAGT ACGAGCCAGA GGACGGCGAG
 CGCACCTCCT TATACTGCTC CTGCTACTCA TGCTCGGTCT CCGCCGCTC
 10951 TACTAAGCGG TGATGTTTCT GATCAGATGA TGCAAGACGC AACGGACCCG
 ATGATTGCC ACTACAAAGA CTAGTCTACT ACGTTCTGCG TTGCCTGGGC
 11001 GCGGTGCGGG CGGCGCTGCA GAGCCAGCCG TCCGGCCTTA ACTCCACGGA
 CGCCACGCCC GCCGCGACGT CTCGGTCGGC AGGCCGGAAT TGAGGTGCCT
 11051 CGACTGGCGC CAGGTCATGG ACCGCATCAT GTCGCTGACT GCGCGCAATC
 GCTGACCGCG GTCCAGTACC TGGCGTAGTA CAGCGACTGA CGCGCGTTAG
 11101 CTGACGCGTT CCGGCAGCAG CCGCAGGCCA ACCGGCTCTC CGCAATTCTG
 GACTGCGCAA GGCCGTCGTC GGCGTCCGGT TGGCCGAGAG GCGTTAAGAC
 11151 GAAGCGGTGG TCCCGGCGCG CGCAAACCCC ACGCACGAGA AGGTGCTGGC
 CTTGCGCCACC AGGGCCGCGC GCGTTTGGGG TCGTGCTCT TCCACGACCG
 11201 GATCGTAAAC GCGCTGGCCG AAAACAGGGC CATCCGGCCC GACGAGGCCG
 CTAGCATTTG CGCGACCGGC TTTTGTCCCG GTAGGCCGGG CTGCTCCGGC
 11251 GCCTGGTCTA CGACGCGCTG CTTGAGCGCG TGGCTCGTTA CAACAGCGGC
 CGGACCAGAT GCTGCGCGAC GAAGTCGCGC ACCGAGCAAT GTTGTCGCGC

Figure 27L

| | | | | | |
|-------|------------|------------|-------------|------------|------------|
| 11351 | GGCGCAGCGT | GAGCGCGCGC | AGCAGCAGGG | CAACCTGGGC | TCCATGGTTG |
| | CCGCGTCGCA | CTCGCGCGCG | TCGTCTGTCC | GTTGGACCCG | AGGTACCAAC |
| 11401 | CACTAAACGC | CTTCCTGAGT | ACACAGCCCCG | CCAACGTGCC | GCGGGGACAG |
| | GTGATTTGCG | GAAGGACTCA | TGTGTCGGGC | GGTTGCACGG | CGCCCCTGTC |
| 11451 | GAGGACTACA | CCAACTTTGT | GAGCGCACTG | CGGCTAATGG | TGACTGAGAC |
| | CTCCTGATGT | GGTTGAAACA | CTCGCGTGAC | GCCGATTACC | ACTGACTCTG |
| 11501 | ACCGCAAAGT | GAGGTGTACC | AGTCTGGGCC | AGACTATTTT | TTCCAGACCA |
| | TGGCGTTTCA | CTCCACATGG | TCAGACCCGG | TCTGATAAAA | AAGGTCTGGT |
| 11551 | GTAGACAAGG | CCTGCAGACC | GTAAACCTGA | GCCAGGCTTT | CAAAAACCTG |
| | CATCTGTTCC | GGACGTCTGG | CATTTGGAAT | CGGTCCGAAA | GTTTTTGAAC |
| 11601 | CAGGGGCTGT | GGGGGGTGCG | GGCTCCCACA | GGCGACCCGG | CGACCGTGTC |
| | GTCCCCGACA | CCCCCCACGC | CCGAGGGTGT | CCGCTGGCGC | GCTGGCACAG |
| 11651 | TAGCTTGCTG | ACGCCCAACT | CGCGCCTGTT | GCTGCTGCTA | ATAGCGCCCT |
| | ATCGAACGAC | TGCGGGTTGA | GCGCGGACAA | CGACGACGAT | TATCGCGGGA |
| 11701 | TCACGGACAG | TGGCAGCGTG | TCCCGGGACA | CATACCTAGG | TCACTTGCTG |
| | AGTGCCGTGC | ACCGTCGCAC | AGGGCCCTGT | GTATGGATCC | AGTGAACGAC |
| 11751 | AACTGTACC | GCGAGGCCAT | AGGTCAGGCG | CATGTGGACG | AGCATACTTT |
| | TGTGACATGG | CGCTCCGGTA | TCCAGTCCGC | GTACACCTGC | TCGTATGAAA |
| 11801 | CCAGGAGATT | ACAAGTGTCA | GCCGCGCGCT | GGGGCAGGAG | GACACGGGCA |
| | GGTCCCTCTA | TGTTACAGT | CGGCGCGCGA | CCCCGTCTTC | CTGTGCCCGT |
| 11851 | GCCTGGAGGC | AACCCTAAAC | TACCTGCTGA | CCAACCGGCG | GCAGAAGATC |
| | CGGACCTCCG | TTGGGATTTG | ATGGACGACT | GGTTGGCCGC | CGTCTTCTAG |
| 11901 | CCCTCGTTGC | ACAGTTTAAA | CAGCGAGGAG | GAGCGCATTT | TGCGCTACGT |
| | GGGAGCAACG | TGTCAAATTT | GTCGCTCCTC | CTCGCGTAAA | ACGCGATGCA |
| 11951 | GCAGCAGAGC | GTGAGCCTTA | ACCTGATGCG | CGACGGGGTA | ACGCCCAGCG |
| | CGTCGTCTCG | CACTCGGAAT | TGGACTACGC | GCTGCCCCAT | TGCGGGTTCG |
| 12001 | TGGCGCTGGA | CATGACCGCG | CGCAACATGG | AACCGGGCAT | GTATGCCTCA |
| | ACCGCGACCT | GTACTGGCGC | GCGTTGTACC | TTGGCCCGTA | CATACGGAGT |
| 12051 | AACCGGCCGT | TTATCAACCG | CCTAATGGAC | TACTTGCAAT | GCGCGGCCGC |
| | TTGGCCGGCA | AATAGTTGGC | GGATTACCTG | ATGAACGTAG | CGCGCCGGCG |
| 12101 | CGTGAACCCC | GAGTATTTCA | CCAATGCCAT | CTTGAACCCG | CACTGGCTAC |
| | GCACCTGGGG | CTCATAAAGT | GGTTACGGTA | GAACCTGGGC | GTGACCGATG |
| 12151 | CGCCCCCTGG | TTTCTACACC | GGGGGATTCG | AGGTGCCCAG | GGGTAACGAT |
| | GCGGGGGACC | AAAGATGTGG | CCCCCTAAGC | TCCACGGGCT | CCCATTGCTA |
| 12201 | GGATTCTCT | GGGACGACAT | AGACGACAGC | GTGTTTTCCC | CGCAACCGCA |
| | CCTAAGGAGA | CCCTGCTGTA | TCTGCTGTGC | CACAAAAGGG | GCGTTGGCGT |

Figure 27 M

12301 AGGAAAGCTT CCGCAGGCCA AGCAGCTTGT CCGATCTAGG CGCTGCGGCC
 TCCTTTTCGA GCGGTCCGGT TCGTCGAACA GGCTAGATCC GCGACGCCGG

12351 CCGCGGTCAG ATGCTAGTAG CCCATTTCCA AGCTTGATAG GGTCTCTTAC
 GGGCCAGTC TACGATCATC GGGTAAAGGT TCGAACTATC CCAGAGAATG

12401 CAGCACTCGC ACCACCCGCC CGCGCCTGCT GGGCGAGGAG GAGTACCTAA
 GTCGTGAGCG TGGTGGGCGG GCGCGGACGA CCCGCTCCTC CTCATGGATT

12451 ACAACTCGCT GCTGCAGCCG CAGCGCGAAA AAAACCTGCC TCCGGCATTT
 TGTTGAGCGA CGACGTCGGC GTCGCGCTTT TTTTGGACGG AGGCCGTAAA

12501 CCCAACAACG GGATAGAGAG CCTAGTGGAC AAGATGAGTA GATGGAAGAC
 GGGTTGTTGC CCTATCTCTC GGATCACCTG TTCTACTCAT CTACCTTCTG

12551 GTACGCGCAG GAGCACAGGG ACGTGCCAGG CCCGCGCCCG CCCACCCGTC
 CATGCGCGTC CTCGTGTCCC TGCACGGTCC GGGCGCGGGC GGGTGGGCAG

12601 GTCAAAGGCA CGACCGTCAG CGGGGTCTGG TGTGGGAGGA CGATGACTCG
 CAGTTTCCGT GCTGGCAGTC GCCCCAGACC ACACCTCCT GCTACTGAGC

12651 GCAGACGACA GCAGCGTCCT GGATTTGGGA GGGAGTGGCA ACCCGTTTGC
 CGTCTGCTGT CGTCGCAGGA CCTAAACCCT CCCTCACCGT TGGGCAAACG

12701 GCACCTTCGC CCCAGGCTGG GGAGAATGTT TAAAAAAA AAAAAGCATG
 CGTGGAAGCG GGGTCCGACC CCTCTTACAA AATTTTTTTT TTTTTCGTAC

12751 ATGCAAAATA AAAAATCAC CAAGGCCATG GCACCGAGCG TTGGTTTTCT
 TACGTTTTAT TTTTGTAGTG GTTCCGGTAC CGTGGCTCGC AACCAAAAGA

12801 TGTATTCCCC TTAGTATGCG GCGCGCGGCG ATGTATGAGG AAGGTCTCTC
 ACATAAGGGG AATCATACGC CGCGCGCCGC TACATACTCC TTCCAGGAGG

12851 TCCCTCCTAC GAGAGTGTGG TGAGCGCGGC GCCAGTGGCG GCGGCGCTGG
 AGGGAGGATG CTCTCACACC ACTCGCGCCG CGGTCACCGC CGCCGCGACC

12901 GTTCTCCCTT CGATGCTCCC CTGGACCCGC CGTTTGTGCC TCCGCGGTAC
 CAAGAGGGAA GCTACGAGGG GACCTGGGCG GCAAACACGG AGGCGCCATG

12951 CTGCGGCCTA CCGGGGGGAG AAACAGCATC CGTTACTCTG AGTTGGCACC
 GACGCCGGAT GGCCCCCTC TTTGTCGTAG GCAATGAGAC TCAACCGTGG

13001 CCTATTGAC ACCACCCGTG TGTACCTGGT GGACAACAAG TCAACGGATG
 GGATAAGCTG TGGTGGGCAC ACATGGACCA CCTGTTGTTC AGTTGCCTAC

13051 TGGCATCCCT GAACTACCAG AACGACCACA GCAACTTTCT GACCACGGTC
 ACCGTAGGGA CTTGATGGTC TTGCTGGTGT CGTTGAAAGA CTGGTGCCAG

13101 ATTCAAAACA ATGACTACAG CCCGGGGGAG GCAAGCACAC AGACCATCAA
 TAAGTTTTGT TACTGATGTC GGGCCCCCTC CGTTCGTGTG TCTGGTAGTT

13151 TCTTGACGAC CGGTCGCACT GGGGCGGCGA CCTGAAAACC ATCCTGCATA
 AGAACTGCTG GCCAGCGTGA CCCC GCCGCT GGACTTTTGG TAGGACGTAT

Figure 27N

| | | | | | |
|-------|-------------|------------|------------|------------|-------------|
| 13251 | CGGGTGATGG | TGTCGCGCTT | GCCTACTAAG | GACAATCAGG | TGGAGCTGAA |
| | GGCCACTACC | ACAGCGCGAA | CGGATGATTC | CTGTTAGTCC | ACCTCGACTT |
| 13301 | ATACGAGTGG | GTGGAGTTCA | CGCTGCCCCG | GGGCAACTAC | TCCGAGACCA |
| | TATGCTCACC | CACCTCAAGT | GCGACGGGCT | CCCGTTGATG | AGGCTCTGGT |
| 13351 | TGACCATAGA | CCTTATGAAC | AACGCGATCG | TGGAGCACTA | CTTGAAAGTG |
| | ACTGGTATCT | GGAATACTTG | TTGCGCTAGC | ACCTCGTGAT | GAACTTTCAC |
| 13401 | GGCAGACAGA | ACGGGGTTCT | GGAAAGCGAC | ATCGGGGTAA | AGTTTGACAC |
| | CCGTCTGTCT | TGCCCCAAGA | CCTTTCGCTG | TAGCCCCATT | TCAAACGTGTG |
| 13451 | CCGCAACTTC | AGACTGGGGT | TTGACCCCGT | CACTGGTCTT | GTCATGCCTG |
| | GGCGTTGAAG | TCTGACCCCA | AACTGGGGCA | GTGACCAGAA | CAGTACGGAC |
| 13501 | GGGTATATAC | AAACGAAGCC | TTCCATCCAG | ACATCATTTT | GCTGCCAGGA |
| | CCCATATATG | TTTGCTTCGG | AAGGTAGGTC | TGTAGTAAAA | CGACGGTCCT |
| 13551 | TGCGGGGTGG | ACTTCACCCA | CAGCCGCTTG | AGCAACTTGT | TGGGCATCCG |
| | ACGCCCCACC | TGAAGTGGGT | GTCGGCGGAC | TCGTTGAACA | ACCCGTAGGC |
| 13601 | CAAGCGGCAA | CCCTTCCAGG | AGGGCTTTAG | GATCACCTAC | GATGATCTGG |
| | GTTCGCCGTT | GGGAAGGTCC | TCCCGAAATC | CTAGTGGATG | CTACTAGACC |
| 13651 | AGGGTGGTAA | CATTCCCGCA | CTGTTGGATG | TGGACGCCTA | CCAGGCGAGC |
| | TCCCACCATT | GTAAGGGCGT | GACAACCTAC | ACCTGCGGAT | GGTCCGCTCG |
| 13701 | TTGAAAGATG | ACACCGAACA | GGGCGGGGGT | GGCGCAGGCG | GCAGCAACAG |
| | AACTTTCTAC | TGTGGCTTGT | CCCGCCCCCA | CCGCGTCCGC | CGTCGTTGTC |
| 13751 | CAGTGGCAGC | GGCGCGGAAG | AGAACTCCAA | CGCGGCAGCC | GCGGCAATGC |
| | GTCACCGTCG | CCGCGCCTTC | TCTTGAGGTT | GCGCCGTCGG | CGCCGTTACG |
| 13801 | AGCCGGTGGA | GGACATGAAC | GATCATGCCA | TTCGCGGCGA | CACCTTTGCC |
| | TCGGCCACCT | CCTGTACTTG | CTAGTACGGT | AAGCGCCGCT | GTGGAAACGG |
| 13851 | ACACGGGCTG | AGGAGAAGCG | CGCTGAGGCC | GAAGCAGCGG | CCGAAGCTGC |
| | TGTGCCCCGAC | TCCTCTTCGC | GCGACTCCGG | CTTCGTCGCC | GGCTTCGACG |
| 13901 | CGCCCCCGCT | GCGCAACCCG | AGGTCGAGAA | GCCTCAGAAG | AAACCGGTGA |
| | GCGGGGGCGA | CGCGTTGGGC | TCCAGCTCTT | CGGAGTCTTC | TTTGCCCACT |
| 13951 | TCAAACCCCT | GACAGAGGAC | AGCAAGAAAC | GCAGTTACAA | CCTAATAAGC |
| | AGTTTGGGGA | CTGTCTCCTG | TCGTTCTTTG | CGTCAATGTT | GGATTATTCTG |
| 14001 | AATGACAGCA | CCTTCACCCA | GTACCGCAGC | TGGTACCTTG | CATACAACCTA |
| | TTACTGTCTG | GGAAGTGGGT | CATGGCGTCG | ACCATGGAAC | GTATGTTGAT |
| 14051 | CGGCGACCCCT | CAGACCGGAA | TCCGCTCATG | GACCCTGCTT | TGCACTCCTG |
| | GCCGCTGGGA | GTCTGGCCTT | AGGCGAGTAC | CTGGGACGAA | ACGTGAGGAC |
| 14101 | ACGTAACCTG | CGGCTCGGAG | CAGGTCTACT | GGTCGTTGCC | AGACATGATG |
| | TGCATTGGAC | GCCGAGCCTC | GTCCAGATGA | CCAGCAACGG | TCTGTACTAC |

Figure 270

14201 GGTGGGCGCC GAGCTGTTGC CCGTGCACTC CAAGAGCTTC TACAACGACC
CCACCCGCGG CTCGACAACG GGCACGTGAG GTTCTCGAAG ATGTTGCTGG

14251 AGGCCGTCTA CTCCCAACTC ATCCGCCAGT TTACCTCTCT GACCCACGTG
TCCGGCAGAT GAGGGTTGAG TAGGCGGTCA AATGGAGAGA CTGGGTGCAC

14301 TTCAATCGCT TTCCCGAGAA CCAGATTTTG GCGCGCCCGC CAGCCCCCAC
AAGTTAGCGA AAGGGCTCTT GGTCTAAAC CGCGCGGGCG GTCGGGGGTG

14351 CATCACCACC GTCAGTGAAA ACGTTCCTGC TCTCACAGAT CACGGGACGC
GTAGTGGTGG CAGTCACTTT TGCAAGGACG AGAGTGTCTA GTGCCCTGCG

14401 TACCGCTGCG CAACAGCATC GGAGGAGTCC AGCGAGTGAC CATTACTGAC
ATGGCGACGC GTTGTCGTAG CCTCCTCAGG TCGCTCACTG GTAATGACTG

14451 GCCAGACGCC GCACCTGCCC CTACGTTTAC AAGGCCCTGG GCATAGTCTC
CGGTCTGCGG CGTGGACGGG GATGCAAATG TTCCGGGACC CGTATCAGAG

14501 GCCGCGCGTC CTATCGAGCC GCACTTTTTG AGCAAGCATG TCCATCCTTA
CGGCGCGCAG GATAGCTCGG CGTGAAAAAC TCGTTCGTAC AGGTAGGAAT

14551 TATCGCCCAG CAATAACACA GGCTGGGGCC TCGGCTTCCC AAGCAAGATG
ATAGCGGGTC GTTATTGTGT CCGACCCCGG ACGCGAAGGG TTCGTTCTAC

14601 TTTGGCGGGG CCAAGAAGCG CTCCGACCAA CACCCAGTGC GCGTGCGCGG
AAACCGCCCC GGTTCCTCGC GAGGCTGGTT GTGGGTACG CGCACGCGCC

14651 GCACTACCGC GCGCCCTGGG GCGCGCACAA ACGCGGCCGC ACTGGGCGCA
CGTGATGGCG CGCGGGACCC CGCGCGTGTT TGCGCCGGCG TGACCCGCGT

14701 CCACCGTCGA TGACGCCATC GACGCGGTGG TGGAGGAGGC GCGCAACTAC
GGTGGCAGCT ACTGCGGTAG CTGCGCCACC ACCTCCTCCG CGCGTTGATG

14751 ACGCCACGC CGCCACCAGT GTCCACAGTG GACGCGGCCA TTCAGACCGT
TGCGGGTGCG GCGGTGGTCA CAGGTGTCAC CTGCGCCGGT AAGTCTGGCA

14801 GGTGCGCGGA GCCCGGCGCT ATGCTAAAT GAAGAGACGG CGGAGGCGCG
CCACGCGCCT CGGGCCGCGA TACGATTTTA CTTCTCTGCC GCCTCCGCGC

14851 TAGCACGTCG CCACCGCCGC CGACCCGGCA CTGCCGCCCA ACGCGCGGCG
ATCGTGCAGC GGTGGCGGCG GCTGGGCCGT GACGGCGGGT TGCGCGCCGC

14901 GCGGCCCTGC TTAACCGCGC ACGTCGCACC GGCCGACGGG CGGCCATGCG
CGCCGGGACG AATTGGCGCG TGCAGCGTGG CCGGCTGCCC GCCGGTACGC

14951 GGCCGCTCGA AGGCTGGCCG CGGGTATTGT CACTGTGCCC CCCAGGTCCA
CCGGCGAGCT TCCGACCGGC GCCCATAACA GTGACACGGG GGGTCCAGGT

15001 GGCGACGAGC GGCCGCCGCA GCAGCCGCGG CCATTAGTGC TATGACTCAG
CCGCTGCTCG CCGGCGGCGT CGTCGGCGCC GGTAATCACG ATACTGAGTC

15051 GGTGCGAGGG GCAACGTGTA TTGGGTGCGC GACTCGGTTA GCGGCCTGCG
CCAGCGTCCC CGTTGCACAT AACCACGCG CTGAGCCAAT CGCCGGACGC

Figure 27P

| | | | | | |
|-------|-------------|------------|------------|------------|-------------|
| 15151 | ACTTAGACTC | GTACTGTTGT | ATGTATCCAG | CGGCGGCGGC | GCGCAACGAA |
| | TGAATCTGAG | CATGACAACA | TACATAGGTC | GCCGCCGCCG | CGCGTTGCTT |
| 15201 | GCTATGTCCA | AGCGCAAAAT | CAAAGAAGAG | ATGCTCCAGG | TCATCGCGCC |
| | CGATACAGGT | TCGCGTTTTA | GTTTCTTCTC | TACGAGGTCC | AGTAGCGCGG |
| 15251 | GGAGATCTAT | GGCCCCCGA | AGAAGGAAGA | GCAGGATTAC | AAGCCCCGAA |
| | CCTCTAGATA | CCGGGGGGCT | TCTTCCTTCT | CGTCCTAATG | TTCGGGGCTT |
| 15301 | AGCTAAAGCG | GGTCAAAAAG | AAAAAGAAAG | ATGATGATGA | TGAACTTGAC |
| | TCGATTTTCG | CCAGTTTTTC | TTTTTCTTTC | TACTACTACT | ACTTGAACTG |
| 15351 | GACGAGGTGG | AACTGCTGCA | CGCTACCGCG | CCCAGGCGAC | GGGTACAGTG |
| | CTGCTCCACC | TTGACGACGT | GCGATGGCGC | GGGTCCGCTG | CCCATGTCAC |
| 15401 | GAAAGGTCGA | CGCGTAAAC | GTGTTTTGCG | ACCCGGCACC | ACCGTAGTCT |
| | CTTTCAGCT | GCGCATTTTG | CACAAAACGC | TGGGCCGTGG | TGGCATCAGA |
| 15451 | TTACGCCCCG | TGAGCGCTCC | ACCCGCACCT | ACAAGCGCGT | GTATGATGAG |
| | AATGCGGGCC | ACTCGCGAGG | TGGGCGTGGA | TGTTCCGCGA | CATACTACTC |
| 15501 | GTGTACGGCG | ACGAGGACCT | GCTTGAGCAG | GCCAACGAGC | GCCTCGGGGA |
| | CACATGCCGC | TGCTCCTGGA | CGAACTCGTC | CGGTTGCTCG | CGGAGCCCCCT |
| 15551 | GTTTGCCTAC | GGAAAGCGGC | ATAAGGACAT | GCTGGCGTTG | CCGCTGGACG |
| | CAAACGGATG | CCTTTCGCCG | TATTCTGTGA | CGACCGCAAC | GGCGACCTGC |
| 15601 | AGGGCAACCC | AACACCTAGC | CTAAAGCCCC | TAACACTGCA | GCAGGTGCTG |
| | TCCCCGTGGG | TTGTGGATCG | GATTTCCGGC | ATTGTGACGT | CGTCCACGAC |
| 15651 | CCCGCGCTTG | CACCGTCCGA | AGAAAAGCGC | GGCCTAAAGC | GCGAGTCTGG |
| | GGGCGCGAAC | GTGGCAGGCT | TCTTTTCGCG | CCGGATTTCG | CGCTCAGACC |
| 15701 | TGACTTGCGA | CCCACCGTGC | AGCTGATGGT | ACCCAAGCGC | CAGCGACTGG |
| | ACTGAACCGT | GGGTGGCACG | TCGACTACCA | TGGGTTCCGC | GTCGCTGACC |
| 15751 | AAGATGTCTT | GGAAAAATG | ACCGTGGAAC | CTGGGCTGGA | GCCCCAGGTC |
| | TTCTACAGAA | CCTTTTTTAC | TGGCACCTTG | GACCCGACCT | CGGGCTCCAG |
| 15801 | CGCGTGCGGC | CAATCAAGCA | GGTGGCGCCG | GGACTGGGCG | TGCAGACCGT |
| | GCGCACGCCG | GTTAGTTCGT | CCACCGCGGC | CCTGACCCGC | ACGTCTGGCA |
| 15851 | GGACGTTTCAG | ATACCCACTA | CCAGTAGCAC | CAGTATTGCC | ACCGCCACAG |
| | CCTGCAAGTC | TATGGGTGAT | GGTCATCGTG | GTCATAACGG | TGGCGGTGTC |
| 15901 | AGGGCATGGA | GACACAAACG | TCCCCGGTTG | CCTCAGCGGT | GGCGGATGCC |
| | TCCCCGTACCT | CTGTGTTTGC | AGGGGCCAAC | GGAGTCGCCA | CCGCCTACGG |
| 15951 | GCGGTGCGAG | CGGTCGCTGC | GGCCGCGTCC | AAGACCTCTA | CGGAGGTGCA |
| | CGCCACGTCC | GCCAGCGACG | CCGGCGCAGG | TTCTGGAGAT | GCCTCCACGT |
| 16001 | AACGGACCCG | TGGATGTTTC | GCGTTTCAGC | CCCCCGGCGC | CCGCGCCGTT |
| | TTGCCCTGGG | ACCTACAAAG | CGCAAAGTCG | GGGGGCCGCG | GGCGCGGCAA |

Figure 27Q

| | | | | | |
|-------|---------------------------|---------------------------|--------------------------|---------------------------|--------------------------|
| 16051 | CGAGGAAGTA GCTCCTTCAT | CGGCGCCGCC GCCGCGGCGG | AGCGCGCTAC TCGCGCGATG | TGCCCCGAATA ACGGGCTTAT | TGCCCTACAT ACGGGATGTA |
| 16101 | CCTTCCATTG GGAAGGTAAC | CGCCTACCCC GCGGATGGGG | CGGCTATCGT GCCGATAGCA | GGCTACACCT CCGATGTGGA | ACCGCCCCAG TGGCGGGGTC |
| 16151 | AAGACGAGCA TTCTGCTCGT | ACTACCCGAC TGATGGGCTG | GCCGAACCAC CGGCTTGTTG | CACTGGAACC GTGACCTTGG | CGCCGCCGCC GCGGCGGCGG |
| 16201 | GTCGCCGTCG CAGCGGCAGC | CCAGCCCCGTG GGTCGGGCAC | CTGGCCCCGA GACCGGGGCT | TTTCCGTGCG AAAGGCACGC | CAGGGTGGCT GTCCACCGA |
| 16251 | CGCGAAGGAG GCGCTTCCTC | GCAGGACCCCT CGTCCTGGGA | GGTGCTGCCA CCACGACGGT | ACAGCGCGCT TGTCGCGCGA | ACCACCCCAG TGGTGGGGTC |
| 16301 | CATCGTTTAA GTAGCAAATT | AAGCCGGTCT TTCGGCCAGA | TTGTGGTTCT AACACCAAGA | TGCAGATATG ACGTCTATAC | GCCCTCACCT CGGGAGTGGA |
| 16351 | GCCGCCCTCCG CGGCGGAGGC | TTTCCCGGTG AAAGGGCCAC | CCGGGATTCC GGCCCTAAGG | GAGGAAGAAT CTCCTTCTTA | GCACCGTAGG CGTGGCATCC |
| 16401 | AGGGGCATGG TCCCCGTACC | CCGGCCACGG GGCCGGTGCC | CCTGACGGGC GGACTGCCCC | GGCATGCGTC CCGTACGCAG | GTGCGCACCA CACGCGTGGT |
| 16451 | CCGGCGGCGG GGCCGCCGCC | CGCGCGTCGC GCGCGCAGCG | ACCGTCGCAT TGGCAGCGTA | GCGCGGCGGT CGCGCCGCCA | ATCCTGCCCC TAGGACGGGG |
| 16501 | TCCTTATTCC AGGAATAAGG | ACTGATCGCC TGACTAGCGG | GCGGCGATTG CGCCGCTAAC | GCGCCGTGCC CGCGGCACGG | CGGAATTGCA GCCTTAACGT |
| 16551 | TCCGTGGCCT AGGCACCGGA | TGCAGGCGCA ACGTCCGCGT | GAGACACTGA CTCTGTGACT | TTAAAAACAA AATTTTGT | GTTGCATGTG CAACGTACAC |
| 16601 | GAAAAATCAA CTTTTAGTT | AATAAAAAGT TTATTTTCA | CTGGACTCTC GACCTGAGAG | ACGCTCGCTT TGCGAGCGAA | GGTCCTGTAA CCAGGACATT |
| 16651 | CTATTTTGTA GATAAAACAT | GAATGGAAGA CTTACCTTCT | CATCAACTTT GTAGTTGAAA | GCGTCTCTGG CGCAGAGACC | CCCCGCGACA GGGGCGCTGT |
| 16701 | CGGCTCGCGC GCCGAGCGCG | CCGTTTCATGG GGCAAGTACC | GAAACTGGCA CTTTGACCGT | AGATATCGGC TCTATAGCCG | ACCAGCAATA TGGTCGTTAT |
| 16751 | TGAGCGGTGG ACTCGCCACC | CGCCTTCAGC GCGGAAGTCG | TGGGGCTCGC ACCCCGAGCG | TGTGGAGCGG ACACCTCGCC | CATTAAAAAT GTAATTTT |
| 16801 | TTCGGTTCCA AAGCCAAGGT | CCGTTAAGAA GGCAATTCTT | CTATGGCAGC GATACCGTCG | AAGGCCTGGA TTCCGGACCT | ACAGCAGCAC TGTCGTCGTG |
| 16851 | AGGCCAGATG TCCGGTCTAC | CTGAGGGATA GACTCCCTAT | AGTTGAAAGA TCAACTTTCT | GCAAAATTTT CGTTTTAAAG | CAACAAAAGG GTTGTTTTCC |
| 16901 | TGGTAGATGG ACCATCTACC | CCTGGCCTCT GGACCGGAGA | GGCATTAGCG CCGTAATCGC | GGGTGGTGGA CCCACCACCT | CCTGGCCAAC GGACCGGTTG |
| 16951 | CAGGCAGTGC GTCCGTCACG | AAAATAAGAT TTTTATTCTA | TAACAGTAAG ATTGTCATT | CTTGATCCCC GAACTAGGGG | GCCCTCCCGT CGGGAGGGCA |

Figure 27R

| | | | | | |
|-------|-------------|-----------------|------------|-----------------|------------|
| 17051 | AAAAGCGTCC | GCGCCCCGAC | AGGGAAGAAA | CTCTGGTGAC | GCAAATAGAC |
| | TTTTCGCAGG | CGCGGGGCTG | TCCCTTCTTT | GAGACCACTG | CGTTTATCTG |
| 17101 | GAGCCTCCCT | CGTACGAGGA | GGCACTAAAG | CAAGGCCTGC | CCACCACCCG |
| | CTCGGAGGGA | GCATGCTCCT | CCGTGATTTC | GTTCCGGACG | GGTGGTGGGC |
| 17151 | TCCCATCGCG | CCCATGGCTA | CCGGAGTGCT | GGGCCAGCAC | ACACCCGTAA |
| | AGGGTAGCGC | GGGTACCGAT | GGCCTCACGA | CCCGGTCGTG | TGTGGGCATT |
| 17201 | CGCTGGACCT | GCCTCCCCCC | GCCGACACCC | AGCAGAAACC | TGTGCTGCCA |
| | GCGACCTGGA | CGGAGGGGGG | CGGCTGTGGG | TCGTCTTTGG | ACACGACGGT |
| 17251 | GGCCCCGACG | CCGTTGTTGT | AACCCGTCTT | AGCCGCGCGT | CCCTGCGCCG |
| | CCGGGCTGGC | GGCAACAACA | TTGGGCAGGA | TCGGC GCGCA | GGGACGCGGC |
| 17301 | CGCCGCCAGC | GGTCCGCGAT | CGTTGCGGCC | CGTAGCCAGT | GGCAACTGGC |
| | GCGGCGGTGC | CCAGGCGCTA | GCAACGCCGG | GCATCGGTCA | CCGTTGACCG |
| 17351 | AAAGCACACT | GAACAGCATC | GTGGGTCTGG | GGGTGCAATC | CCTGAAGCGC |
| | TTTCGTGTGA | CTTGTCGTAG | CACCCAGACC | CCCACGTTAG | GGACTTCGCG |
| 17401 | CGACGATGCT | TCTGATAGCT | AACGTGTCTG | ATGTGTGTCA | TGTATGCGTC |
| | GCTGCTACGA | AGACTATCGA | TTGCACAGCA | TACACACAGT | ACATACGCAG |
| 17451 | CATGTCGCCG | CCAGAGGAGC | TGCTGAGCCG | CCGCGCGCCC | GCTTTCCAAG |
| | GTACAGCGGC | GGTCTCCTCG | ACGACTCGGC | GGCGCGCGGG | CGAAAGGTTT |
| 17501 | ATGGCTACCC | CTTCGATGAT | GCCGCAGTGG | TCTTACATGC | ACATCTCGGG |
| | TACCGATGGG | GAAGCTACTA | CGGCGTCACC | AGAATGTACG | TGTAGAGCCC |
| 17551 | CCAGGACGCC | TCGGAGTACC | TGAGCCCCGG | GCTGGTGCCG | TTTGCCCCGG |
| | GGTCTGCGG | AGCCTCATGG | ACTCGGGGCC | CGACCACGTC | AAACGGGGCG |
| 17601 | CCACCGAGAC | GTA T T C A G C | CTGAATAACA | AGTTTAGAAA | CCCCACGGTG |
| | GGTGGCTCTG | CATGAAGTCG | GACTTATTGT | TCAAATCTTT | GGGGTGCCAC |
| 17651 | GCGCCTACGC | ACGACGTGAC | CACAGACCGG | TCCCAGCGTT | TGACGCTGCG |
| | CGCGGATGCG | TGCTGCACTG | GTGTCTGGCC | AGGGTCGCAA | ACTGCGACGC |
| 17701 | GTTTCATCCCT | GTGGACCGTG | AGGATACTGC | GTA T C G T A C | AAGGCGCGGT |
| | CAAGTAGGGA | CACCTGGCAC | TCCTATGACG | CATGAGCATG | TTCCGCGCCA |
| 17751 | TCACCCTAGC | TGTGGGTGAT | AACCGTGTGC | TGGACATGGC | TTCCACGTAC |
| | AGTGGGATCG | ACACCCACTA | TTGGCACACG | ACCTGTACCG | AAGGTGCATG |
| 17801 | TTTGACATCC | GCGGCGTGCT | GGACAGGGGC | CCTACTTTTA | AGCCCTACTC |
| | AAACTGTAGG | CGCCGCACGA | CCTGTCCCCG | GGATGAAAAT | TCGGGATGAG |
| 17851 | TGGCACTGCC | TACAACGCCC | TGGCTCCCAA | GGGTGCCCCA | AATCCTTGCG |
| | ACCGTGACGG | ATGTTGCGGG | ACCGAGGGTT | CCCACGGGGT | TTAGGAACGC |
| 17901 | AATGGGATGA | AGCTGCTACT | GCTCTTGAAA | TAAACCTAGA | AGAAGAGGAC |
| | TTACCCTACT | TCGACGATGA | CGAGAACTTT | ATTTGGATCT | TCTTCTCCTG |

Figure 275

17951 GATGACAACG AAGACGAAGT AGACGAGCAA GCTGAGCAGC AAAAACTCA
CTACTGTTGC TTCTGCTTCA TCTGCTCGTT CGACTCGTCG TTTTTTGAGT

18001 CGTATTTGGG CAGGCGCCTT ATTCTGGTAT AAATATTACA AAGGAGGGTA
GCATAAACCC GTCCGCGGAA TAAGACCATA TTTATAATGT TTCCTCCCAT

18051 TTCAAATAGG TGTCTGAAGT CAAACACCTA AATATGCCGA TAAACATTT
AAGTTTATCC ACAGCTTCCA GTTTGTGGAT TTATACGGCT ATTTTGTA

18101 CAACCTGAAC CTCAAATAGG AGAATCTCAG TGGTACGAAA CAGAAATTAA
GTTGGACTTG GAGTTTATCC TCTTAGAGTC ACCATGCTTT GTCTTTAATT

18151 TCATGCAGCT GGGAGAGTCC TAAAAAGAC TACCCCAATG AAACCATGTT
AGTACGTCGA CCCTCTCAGG ATTTTTCCTG ATGGGGTTAC TTTGGTACAA

18201 ACGGTTTCATA TGCAAAACCC ACAAATGAAA ATGGAGGGCA AGGCATTCTT
TGCCAAGTAT ACGTTTTGGG TGTTTACTTT TACCTCCCGT TCCGTAAGAA

18251 GTAAAGCAAC AAAATGGAAA GCTAGAAAGT CAAGTGGAAA TGCAATTTTT
CATTTTCGTTG TTTTACCTTT CGATCTTTCA GTTCACCTTT ACGTTAAAAA

18301 CTCAACTACT GAGGCAGCCG CAGGCAATGG TGATAACTTG ACTCCTAAAG
GAGTTGATGA CTCCGTCGGC GTCCGTTACC ACTATTGAAC TGAGGATTTT

18351 TGGTATTGTA CAGTGAAGAT GTAGATATAG AAACCCAGCA CACTCATATT
ACCATAACAT GTCACCTCTA CATCTATATC TTTGGGGTCT GTGAGTATAA

18401 TCTTACATGC CCACTATTAA GGAAGGTAAC TCACGAGAAC TAATGGGCCA
AGAATGTACG GGTGATAATT CCTTCCATTG AGTGCTCTTG ATTACCCGGT

18451 ACAATCTATG CCCAACAGGC CTAATTACAT TGCTTTTAGG GACAATTTTA
TGTTAGATAC GGGTTGTCCG GATTAATGTA ACGAAAATCC CTGTTAAAT

18501 TTGGTCTAAT GTATTACAAC AGCACGGGTA ATATGGGTGT TCTGGCGGGC
AACCAGATTA CATAATGTTG TCGTGCCCAT TATACCCACA AGACCGCCCG

18551 CAAGCATCGC AGTTGAATGC TGTTGTAGAT TTGCAAGACA GAAACACAGA
GTTTCGTAGCG TCAACTTACG ACAACATCTA AACGTTCTGT CTTTGTGTCT

18601 GCTTTCATAC CAGCTTTTGC TTGATTCCAT TGGTGATAGA ACCAGGTACT
CGAAAGTATG GTCGAAAACG AACTAAGGTA ACCACTATCT TGGTCCATGA

18651 TTTCTATGTG GAATCAGGCT GTTGACAGCT ATGATCCAGA TGTTAGAATT
AAAGATACAC CTTAGTCCGA CAACTGTCGA TACTAGGTCT ACAATCTTAA

18701 ATTGAAAATC ATGGAAGTGA AGATGAACTT CCAAATTACT GCTTTCCTACT
TAACCTTTAG TACCTTGACT TCTACTTGAA GGTTTAATGA CGAAAGGTGA

18751 GGGAGGTGTG ATTAATACAG AGACTCTTAC CAAGGTAAAA CCTAAACAG
CCCTCCACAC TAATTATGTC TCTGAGAATG GTTCCATTTT GGATTTTGTC

18801 GTCAGGAAAA TGGATGGGAA AAAGATGCTA CAGAATTTTC AGATAAAAAAT
CAGTCCTTTT ACCTACCCTT TTTCTACGAT GTCTTAAAAG TCTATTTTAA

18851 GAAATAAGAG TTGGAAATAA TTTTGCCATG GAAATCAATC TAAATGCCAA
CTTTATTCTC AACCTTTATT AAAACGGTAC CTTTAGTTAG ATTTACGGTT

Figure 27T

18951 AGCTAAAGTA CAGTCCTTCC AACGTAAAAA TTTCTGATAA CCCAAACACC
TCGATTTCAT GTCAGGAAGG TTGCATTTTT AAAGACTATT GGGTTTGTGG

19001 TACGACTACA TGAACAAGCG AGTGGTGGCT CCCGGGCTAG TGGACTGCTA
ATGCTGATGT ACTTGTTTCGC TCACCACCGA GGGCCCCGATC ACCTGACGAT

19051 CATTAACTTT GGAGCACGCT GGTCCCTTGA CTATATGGAC AACGTCAACC
GTAATTGGAA CCTCGTGCGA CCAGGGAAC TATATACCTG TTGCAGTTGG

19101 CATTTAACCA CCACCGCAAT GCTGGCCTGC GCTACCGCTC AATGTTGCTG
GTAAATTGGT GGTGGCGTTA CGACCGGACG CGATGGCGAG TTACAACGAC

19151 GGCAATGGTC GCTATGTGCC CTTCCACATC CAGGTGCCTC AGAAGTTCTT
CCGTTACCAG CGATACACGG GAAGGTGTAG GTCCACGGAG TCTTCAAGAA

19201 TGCCATTAAA AACCTCCTTC TCCTGCCGGG CTCATACACC TACGAGTGGA
ACGGTAATTT TTGGAGGAAG AGGACGGCCC GAGTATGTGG ATGCTCACCT

19251 ACTTCAGGAA GGATGTTAAC ATGGTTCTGC AGAGCTCCCT AGGAAATGAC
TGAAGTCCTT CCTACAATTG TACCAAGACG TCTCGAGGGA TCCTTTACTG

19301 CTAAGGGTTG ACGGAGCCAG CATTAAAGTTT GATAGCATTT GCCTTTACGC
GATTCCCAAC TGCCTCGGTC GTAATTCAAA CTATCGTAAA CGGAAATGCG

19351 CACCTTCTTC CCCATGGCCC ACAACACCGC CTCCACGCTT GAGGCCATGC
GTGGAAGAAG GGGTACCGGG TGTTGTGGCG GAGGTGCGAA CTCCGGTACG

19401 TTAGAAACGA CACCAACGAC CAGTCCTTTA ACGACTATCT CTCCGCCGCC
AATCTTTGCT GTGGTTGCTG GTCAGGAAAT TGCTGATAGA GAGGCGGCGG

19451 AACATGCTCT ACCCTATACC CGCCAACGCT ACCAACGTGC CCATATCCAT
TTGTACGAGA TGGGATATGG GCGGTTGCGA TGTTGTCACG GGTATAGGTA

19501 CCCCTCCCGC AACTGGGCGG CTTTCCGCGG CTGGGCCTTC ACGCGCCTTA
GGGGAGGGCG TTGACCCGCC GAAAGGCGCC GACCCGGAAG TGCGCGGAAT

19551 AGACTAAGGA AACCCCATCA CTGGGCTCGG GCTACGACCC TTATTACACC
TCTGATTCCT TTGGGGTAGT GACCCGAGCC CGATGCTGGG AATAATGTGG

19601 TACTCTGGCT CTATACCCTA CCTAGATGGA ACCTTTTACC TCAACCACAC
ATGAGACCGA GATATGGGAT GGATCTACCT TGGAAAATGG AGTTGGTGTG

19651 CTTTAAGAAG GTGGCCATTA CCTTTGACTC TTCTGTCAGC TGGCCTGGCA
GAAATTCTTC CACCGGTAAT GGAAACTGAG AAGACAGTCG ACCGGACCGT

19701 ATGACCGCCT GCTTACCCCC AACGAGTTTG AAATTAAGCG CTCAGTTGAC
TACTGGCGGA CGAATGGGGG TTGCTCAAAC TTTAATTTCG GAGTCAACTG

19751 GGGGAGGGTT ACAACGTTGC CCAGTGTAAC ATGACCAAAG ACTGGTTCTT
CCCCTCCCAA TGTTGCAACG GGTACATTG TACTGGTTTC TGACCAAGGA

19801 GGTACAAATG CTAGCTAACT ATAACATTGG CTACCAGGGC TTCTATATCC
CCATGTTTAC GATCGATTGA TATTGTAACC GATGGTCCCG AAGATATAGG

Figure 274

19851 CAGAGAGCTA CAAGGACCGC ATGTACTCCT TCTTTAGAAA CTTCCAGCCC
GTCTCTCGAT GTTCCTGGCG TACATGAGGA AGAAATCTTT GAAGGTCGGG

19901 ATGAGCCGTC AGGTGGTGGA TGATACTAAA TACAAGGACT ACCAACAGGT
TACTCGGCAG TCCACCACCT ACTATGATTT ATGTTCTCTGA TGGTTGTCCA

19951 GGGCATCCTA CACCAACACA ACAACTCTGG ATTTGTTGGC TACCTTGCCC
CCCGTAGGAT GTGGTTGTGT TGTTGAGACC TAAACAACCG ATGGAACGGG

20001 CCACCATGCG CGAAGGACAG GCCTACCCTG CTAACCTCCC CTATCCGCTT
GGTGGTACGC GCTTCCTGTC CGGATGGGAC GATTGAAGGG GATAGGCGAA

20051 ATAGGCAAGA CCGCAGTTGA CAGCATTACC CAGAAAAAGT TTCTTTGCGA
TATCCGTTCT GCGCTCAACT GTCGTAATGG GTCTTTTTTCA AAGAAACGCT

20101 TCGCACCCCTT TGGCGCATCC CATTCTCCAG TAACTTTATG TCCATGGGCG
AGCGTGGGAA ACCGCGTAGG GTAAGAGGTC ATTGAAATAC AGGTACCCCG

20151 CACTCACAGA CCTGGGCCAA AACCTTCTCT ACGCCAACTC CGCCCACGCG
GTGAGTGTCT GGACCCGGTT TTGGAAGAGA TCGGTTGAG GCGGGTGCGC

20201 CTAGACATGA CTTTTGAGGT GGATCCCATG GACGAGCCCA CCCTTCTTTA
GATCTGTACT GAAAACTCCA CCTAGGGTAC CTGCTCGGGT GGGAAGAAAT

20251 TGTTTTGTTT GAAGTCTTTG ACGTGGTCCG TGTGCACCAG CCGCACCGCG
ACAAAACAAA CTTCAGAAAC TGCACCAGGC ACACGTGGTC GCGGTGGCGC

20301 GCGTCATCGA AACCGTGTAC CTGCGCACGC CCTTCTCGGC CGGCAACGCC
CGCAGTAGCT TTGGCACATG GACGCGTGCG GGAAGAGCCG GCCGTTGCGG

20351 ACAACATAAA GAAGCAAGCA ACATCAACAA CAGCTGCCGC CATGGGCTCC
TGTTGTATTT CTTCGTTTCG TGTAGTTGTT GTCGACGGCG GTACCCGAGG

20401 AGTGAGCAGG AACTGAAAGC CATTGTCAA GATCTTGGTT GTGGGCCATA
TCACTCGTCC TTGACTTTCG GTAACAGTTT CTAGAACCAA CACCCGGTAT

20451 TTTTTTGGGC ACCTATGACA AGCGCTTTCC AGGCTTTGTT TCTCCACACA
AAAAAACCCG TGGATACTGT TCGCGAAAGG TCCGAAACAA AGAGGTGTGT

20501 AGCTCGCCTG CGCCATAGTC AATACGGCCG GTCGCGAGAC TGGGGGCGTA
TCGAGCGGAC GCGGTATCAG TTATGCCGGC CAGCGCTCTG ACCCCCGCAT

20551 CACTGGATGG CCTTTGCCTG GAACCCGCAC TCAAAAACAT GCTACCTCTT
GTGACCTACC GGAAACGGAC CTTGGGCGTG AGTTTTTGTA CGATGGAGAA

20601 TGAGCCCTTT GGCTTTTCTG ACCAGCGACT CAAGCAGGTT TACCAGTTTG
ACTCGGGAAC CCGAAAAGAC TGGTCGCTGA GTTCGTCCAA ATGGTCAAAC

20651 AGTACGAGTC ACTCCTGCGC CGTAGCGCCA TTGCTTCTTC CCCCAGCCGC
TCATGCTCAG TGAGGACGCG GCATCGCGGT AACGAAGAAG GGGGCTGGCG

20701 TGTATAACGC TGGAAAAGTC CACCCAAAGC GTACAGGGGC CCAACTCGGC
ACATATTGCG ACCTTTTCAG GTGGGTTTCG CATGTCCCCG GGTTGAGCCG

20751 CGCCTGTGGA CTATTCTGCT GCATGTTTCT CCACGCCTTT GCCAACTGGC
GCGGACACCT GATAAGACGA CGTACAAAGA GGTGCGGAAA CGGTTGACCG

Figure 27 V.

20851 CCCAACTCCA TGCTCAACAG TCCCCAGGTA CAGCCCACCC TCGGTCGCAA
GGGTTGAGGT ACGAGTTGTC AGGGGTCCAT GTCGGGTGGG ACGCAGCGTT

20901 CCAGGAACAG CTCTACAGCT TCCTGGAGCG CCACTCGCCC TACTTCCGCA
GGTCCTTGTC GAGATGTGCA AGGACCTCGC GGTGAGCGGG ATGAAGGCGT

20951 GCCACAGTGC GCAGATTAGG AGCGCCACTT CTTTTTGTCA CTTGAAAAAC
CGGTGTCACG CGTCTAATCC TCGCGGTGAA GAAAAACAGT GAACTTTTTG

21001 ATGTAAAAAT AATGTACTAG AGACACTTTC AATAAAGGCA AATGCTTTTA
TACATTTTTA TTACATGATC TCTGTGAAAG TTATTTCCGT TTACGAAAAAT

21051 TTTGTACACT CTCGGGTGAT TATTTACCCC CACCCTTGCC GTCTGCGCCG
AAACATGTGA GAGCCCACTA ATAAATGGGG GTGGGAACGG CAGACGCGGC

21101 TTTAAAAATC AAAGGGGTTC TGCCGCGCAT CGCTATGCGC CACTGGCAGG
AAATTTTTAG TTTCCCAAG ACGGCGCGTA GCGATACGG GTGACCGTCC

21151 GACACGTTGC GATACTGGTG TTTAGTGCTC CACTTAAACT CAGGCACAAC
CTGTGCAACG CTATGACCAC AAATCACGAG GTGAATTTGA GTCCGTGTTG

21201 CATCCGCGGC AGCTCGGTGA AGTTTTCACT CCACAGGCTG CGCACCATCA
GTAGGCGCCG TCGAGCCACT TCAAAAGTGA GGTGTCCGAC GCGTGGTAGT

21251 CCAACGCGTT TAGCAGGTCG GCGCCGATA TCTTGAAGTC GCAGTTGGGG
GGTTGCGCAA ATCGTCCAGC CCGCGGCTAT AGAACTTCAG CGTCAACCCC

21301 CCTCCGCCCT GCGCGCGCGA GTTGCGATAC ACAGGGTTGC AGCACTGGAA
GGAGGCGGGA CGCGCGCGCT CAACGCTATG TGTCCCAACG TCGTGACCTT

21351 CACTATCAGC GCCGGGTGGT GCACGCTGGC CAGCACGCTC TTGTGCGAGA
GTGATAGTCG CGGCCCACCA CGTGCGACCG GTCGTGCGAG AACAGCCTCT

21401 TCAGATCCGC GTCCAGGTCC TCCGCGTTGC TCAGGGCGAA CGGAGTCAAC
AGTCTAGGCG CAGGTCCAGG AGGCGCAACG AGTCCCCTT GCCTCAGTTG

21451 TTTGGTAGCT GCCTTCCCAA AAAGGGCGCG TGCCCAGGCT TTGAGTTGCA
AAACCATCGA CGGAAGGGTT TTTCCGCGC ACGGGTCCGA AACTCAACGT

21501 CTCGCACCGT AGTGGCATCA AAAGGTGACC GTGCCCGGTC TGGGCGTTAG
GAGCGTGGCA TCACCGTAGT TTTCCACTGG CACGGGCCAG ACCCGCAATC

21551 GATACAGCGC CTGCATAAAA GCCTTGATCT GCTTAAAAGC CACCTGAGCC
CTATGTCGCG GACGTATTTT CGGAACTAGA CGAATTTTCG GTGGACTCGG

21601 TTTGCGCCTT CAGAGAAGAA CATGCCGCAA GACTTGCCGG AAAACTGATT
AAACGCGGAA GTCTCTTCTT GTACGGCGTT CTGAACGGCC TTTTGAATAA

21651 GGCCGGACAG GCCGCGTCGT GCACGCAGCA CTTGCGTCG GTGTTGGAGA
CCGGCCTGTC CGGCGCAGCA CGTGCGTCGT GGAACGCAGC CACAACCTCT

21701 TCTGCACCAC ATTTGCGCCC CACCGGTTCT TCACGATCTT GGCCTTGCTA
AGACGTGGTG TAAAGCCGGG GTGGCCAAGA AGTGCTAGAA CCGGAACGAT

Figure 27 W

21801 AATCACGTGC TCCTTATTTA TCATAATGCT TCCGTGTAGA CACTTAAGCT
 TTAGTGCACG AGGAATAAAT AGTATTACGA AGGCACATCT GTGAATTCGA
 21851 CGCCTTCGAT CTCAGCGCAG CGGTGCAGCC ACAACGCGCA GCCCCTGGGC
 GCGGAAGCTA GAGTCGCGTC GCCACGTCGG TGTTCGCGCT CGGGCACCCG
 21901 TCGTGATGCT TGTAGGTCAC CTCTGCAAAC GACTGCAGGT ACGCCTGCAG
 AGCACTACGA ACATCCAGTG GAGACGTTTG CTGACGTCCA TCGGGACGTC
 21951 GAATCGCCCC ATCATCGTCA CAAAGGTCTT GTTGCTGGTG AAGGTCAGCT
 CTTAGCGGGG TAGTAGCAGT GTTTCAGAA CAACGACCAC TTCCAGTCGA
 22001 GCAACCCGCG GTGCTCCTCG TTCAGCCAGG TCTTGCATAC GGCCGCCAGA
 CGTTGGGCGC CACGAGGAGC AAGTCGGTCC AGAACGTATG CCGGCGGTCT
 22051 GCTTCCACTT GGTCAGGCAG TAGTTTGAAG TTCGCCTTTA GATCGTTATC
 CGAAGGTGAA CCAGTCCGTC ATCAAACCTT AAGCGGAAAT CTAGCAATAG
 22101 CACGTGGTAC TTGTCCATCA GCGCGCGCGC AGCCTCCATG CCCTTCTCCC
 GTGCACCATG AACAGGTAGT CGCGCGCGCG TCGGAGGTAC GGGGAAGAGG
 22151 ACGCAGACAC GATCGGCACA CTCAGCGGGT TCATCACCGT AATTTCACTT
 TCGCTCTGTG CTAGCCGTGT GAGTCGCCCA AGTAGTGGCA TTAAAGTGAA
 22201 TCCGCTTCGC TGGGCTCTTC CTCTTCTCT TCGCTCCGCA TACCACGCGC
 AGGCGAAGCG ACCCGAGAAG GAGAAGGAGA ACGCAGGCGT ATGGTGC GCG
 22251 CACTGGGTCG TCTTCATTCA GCCGCCGCAC TGTGCGCTTA CCTCCTTTGC
 GTGACCCAGC AGAAGTAAGT CGGCGGCGTG ACACGCGAAT GGAGGAAACG
 22301 CATGCTTGAT TAGCACCAGT GGGTTGCTGA AACCCACCAT TTGTAGCGCC
 GTACGAACATA ATCGTGGCCA CCCAACGACT TTGGGTGGTA AACATCGCGG
 22351 ACATCTTCTC TTTCTTCTC GCTGTCCACG ATTACCTCTG GTGATGGCGG
 TGTAGAAGAG AAAGAAGGAG CGACAGGTGC TAATGGAGAC CACTACCGCC
 22401 GCGCTCGGGC TTGGGAGAAG GCGCTTCTT TTTCTTCTTG GCGGCAATGG
 CGCGAGCCCG AACCTCTTC CCGCGAAGAA AAAGAAGAAC CCGCGTTACC
 22451 CCAAATCCGC CGCCGAGGTC GATGGCCGCG GGCTGGGTGT GCGCGGCACC
 GGTTTAGGCG GCGGCTCCAG CTACCGGCGC CCGACCCACA CGCGCCGTGG
 22501 AGCGCGTCTT GTGATGAGTC TTCCTCGTCC TCGGACTCGA TACGCCGCCT
 TCGCGCAGAA CACTACTCAG AAGGAGCAGG AGCCTGAGCT ATGCGGCGGA
 22551 CATCCGCTTT TTTGGGGGCG CCCGGGGAGG CGGCGGCGAC GGGGACGGGG
 GTAGGCGAAA AAACCCCGC GGGCCCTCC GCCGCGCTG CCCCTGCCCC
 22601 ACGACACGTC CTCCATGGTT GGGGGACGTC GCGCCGCACC GCGTCCGCGC
 TGCTGTGCAG GAGGTACCAA CCCCTGCAG CGCGGCGTGG CGCAGGCGCG
 22651 TCGGGGGTGG TTTCGCGCTG CTCCTCTTCC CGACTGGCCA TTTCTTCTC
 AGCCCCCACC AAAGCGCGAC GAGGAGAAGG GCTGACCGGT AAAGGAAGAG

Figure 27 X

22751 CCGCCCCCTC TGAGTTCGCC ACCACCGCCT CCACCGATGC CGCCAACGCG
GGCGGGGGAG ACTCAAGCGG TGGTGGCGGA GGTGGCTACG GCGGTTGCGC

22801 CCTACCACCT TCCCCGTCGA GGCACCCCGG CTTGAGGAGG AGGAAGTGAT
GGATGGTGGA AGGGGCAGCT CCGTGGGGGC GAACTCCTCC TCCTTCACTA

22851 TATCGAGCAG GACCCAGGTT TTGTAAGCGA AGACGACGAG GACCGCTCAG
ATAGCTCGTC CTGGGTCCAA AACATTGCTC TCTGCTGCTC CTGGCGAGTC

22901 TACCAACAGA GGATAAAAAG CAAGACCAGG ACAACGCAGA GGCAAACGAG
ATGGTTGTCT CCTATTTTTC GTTCTGGTCC TGTTGCGTCT CCGTTTGCTC

22951 GAACAAGTCG GCGGGGGGGA CGAAAGGCAT GCGGACTACC TAGATGTGGG
CTTGTTTCAGC CCGCCCCCTT GCTTTCCGTA CCGCTGATGG ATCTACACCC

23001 AGACGACGTG CTGTTGAAGC ATCTGCAGCG CCAGTGCGCC ATTATCTGCG
TCTGCTGCAC GACAACTTCG TAGACGTGCG GGTACGCGG TAATAGACGC

23051 ACGCGTTGCA AGAGCGCAGC GATGTGCCCC TCGCCATAGC GGATGTCAGC
TGCGCAACGT TCTCGCGTCG CTACACGGGG AGCGGTATCG CCTACAGTCG

23101 CTTGCCTACG AACGCCACCT ATTCTCACCG CGCGTACCCC CCAAACGCCA
GAACGGATGC TTGCGGTGGA TAAGAGTGGC GCGCATGGGG GGTGTCGGT

23151 AGAAAACGGC ACATGCGAGC CCAACCCGCG CCTCAACTTC TACCCCGTAT
TCTTTTGCCG TGTACGCTCG GGTGCGGCGC GGAGTTGAAG ATGGGGGCATA

23201 TTGCCGTGCC AGAGGTGCTT GCCACCTATC ACATCTTTTT CCAAACTGC
AACGGCACGG TCTCCACGAA CGGTGGATAG TGTAGAAAAA GGTGTTGACG

23251 AAGATACCCC TATCCTGCCG TGCCAACCGC AGCCGAGCGG ACAAGCAGCT
TTCTATGGGG ATAGGACGGC ACGGTTGGCG TCGGCTCGCC TGTTGCTCGA

23301 GGCCTTGCGG CAGGGCGCTG TCATACCTGA TATCGCCTCG CTCAACGAAG
CCGGAACGCC GTCCCGCGAC AGTATGGACT ATAGCGGAGC GAGTTGCTTC

23351 TGCCAAAAAT CTTTGAGGGT CTTGGACGCG ACGAGAAGCG CGCGGCAAAC
ACGGTTTTTA GAAACTCCCA GAACCTGCGC TGCTCTTCGC GCGCCGTTTG

23401 GCTCTGCAAC AGGAAAAACAG CGAAAAATGAA AGTCACTCTG GAGTGTGGT
CGAGACGTTG TCCTTTTGTC GCTTTTACTT TCAGTGAGAC CTCACAACCA

23451 GGAACCTGAG GGTGACAACG CGCGCCTAGC CGTACTAAAA CGCAGCATCG
CCTTGAGCTC CCACTGTTGC GCGCGGATCG GCATGATTTT GCGTCGTAGC

23501 AGGTCACCCA CTTTGCTTAC CCGGCACTTA ACCTACCCCC CAAGGTCATG
TCCAGTGGGT GAAACGGATG GGCCGTGAAT TGGATGGGGG GTTCCAGTAC

23551 AGCACAGTCA TGAGTGAGCT GATCGTGCGC CGTGCGCAGC CCCTGGAGAG
TCGTGTCAGT ACTCACTCGA CTAGCACGCG GCACGCGTCG GGGACCTCTC

23601 GGATGCAAAT TTGCAAGAAC AAACAGAGGA GGGCCTACCC GCAGTTGGCG
CCTACGTTTA AACGTTCTTG TTTGTCTCCT CCCGGATGGG CGTCAACCGC

Figure 27 Y

23701 GAGCGACGCA AACTAATGAT GGCCGCAGTG CTCGTTACCG TGGAGCTTGA
CTCGCTGCGT TTGATTACTA CCGGCGTCAC GAGCAATGGC ACCTCGAACT

23751 GTGCATGCAG CGGTTCTTTG CTGACCCGGA GATGCAGCGC AAGCTAGAGG
CACGTACGTC GCCAAGAAAC GACTGGGCCT CTACGTGCGG TTCGATCTCC

23801 AAACATTGCA CTACACCTTT CGACAGGGCT ACGTACGCCA GGCCTGCAAG
TTTGTAACTG GATGTGGAAG GCTGTCCCGA TGCATGCGGT CCGGACGTTC

23851 ATCTCCAACG TGGAGCTCTG CAACCTGGTC TCCTACCTTG GAATTTTGCA
TAGAGGTTGC ACCTCGAGAC GTTGGACCAG AGGATGGAAC CTTAAAACGT

23901 CGAAAACCGC CTTGGGCAAA ACGTGCTTCA TTCCACGCTC AAGGGCGAGG
GCTTTTGCGG GAACCCGTTT TGCACGAAGT AAGGTGCGAG TTCCCCTCC

23951 CGCGCCGCGA CTACGTCCGC GACTGCGTTT ACTTATTTCT ATGCTACACC
GCGCGGCGCT GATGCAGGCG CTGACGCAA TGAATAAAGA TACGATGTGG

24001 TGGCAGACGG CCATGGGCGT TTGGCAGCAG TGCTTGGAGG AGTGCAACCT
ACCGTCTGCC GGTACCCGCA AACCCTCGTC ACGAACCTCC TCACGTTGGA

24051 CAAGGAGCTG CAGAACTGC TAAAGCAAAA CTTGAAGGAC CTATGGACGG
GTTCTTCGAC GTCTTTGACG ATTTCTGTTT GAACTTCCTG GATACCTGCC

24101 CCTTCAACGA GCGCTCCGTG GCCGCGCACC TGGCGGACAT CATTTTCCCC
GGAAGTTGCT CGCGAGGCAC CGGCGCGTGG ACCGCTGTGA GTAAAAGGGG

24151 GAACGCCTGC TTAACCCT GCAACAGGGT CTGCCAGACT TCACCAGTCA
CTTGCGGACG AATTTTGGA CGTTGTCCCA GACGGTCTGA AGTGGTCAGT

24201 AAGCATGTTG CAGAACTTTA GGAACCTTAT CCTAGAGCGC TCAGGAATCT
TTCGTACAAC GTCTTGAAAT CTTGAAATA GGATCTCGCG AGTCCTTAGA

24251 TGCCCGCCAC CTGCTGTGCA CTTCTAGCG ACTTTGTGCC CATTAAGTAC
ACGGGCGGTG GACGACACGT GAAGGATCGC TGAAACACGG GTAATTCTATG

24301 CGCGAATGCC CTCCGCCGCT TTGGGGCCAC TGCTACCTTC TGCAGCTAGC
GCGCTTACGG GAGGCGGCGA AACCCCGGTG ACGATGGAAG ACGTCGATCG

24351 CAACTACCTT GCCTACCACT CTGACATAAT GGAAGACGTG AGCGGTGACG
GTTGATGGAA CGGATGGTGA GACTGTATTA CTTCTGCAC TCGCCACTGC

24401 GTCTACTGGA GTGTCACTGT CGCTGCAACC TATGCACCCC GCACCGCTCC
CAGATGACCT CACAGTGACA GCGACGTTGG ATACGTGGGG CGTGGCGAGG

24451 CTGGTTTGCA ATTCGCAGCT GCTTAACGAA AGTCAAATTA TCGGTACCTT
GACCAAACGT TAAGCGTCGA CGAATTGCTT TCAGTTTAAAT AGCCATGGAA

24501 TGAGCTGCAG GGTCCCTCGC CTGACGAAAA GTCCGCGGCT CCGGGGTTGA
ACTCGACGTC CCAGGGAGCG GACTGCTTTT CAGGCGCCGA GGCCCCAACT

24551 AACTCACTCC GGGGCTGTGG ACGTCGGCTT ACCTTCGCAA ATTTGTACCT
TTGAGTGAGG CCCCACACC TGCAGCCGAA TGGAAGCGTT TAAACATGGA

Figure 272

| | | | | | |
|-------|------------|------------|------------|------------|------------|
| 24601 | GAGGACTACC | ACGCCCACGA | GATTAGGTTC | TACGAAGACC | AATCCCCGCC |
| | CTCCTGATGG | TGCGGGTGCT | CTAATCCAAG | ATGCTTCTGG | TTAGGGCGGG |
| 24651 | GCCTAATGCG | GAGCTTACCG | CCTGCGTCAT | TACCCAGGGC | CACATTCTTG |
| | CGGATTACGC | CTCGAATGGC | GGACGCAGTA | ATGGGTCCCG | GTGTAAGAAC |
| 24701 | GCCAATTGCA | AGCCATCAAC | AAAGCCCGCC | AAGAGTTTCT | GCTACGAAAG |
| | CGGTTAACGT | TCGGTAGTTG | TTTCGGGCGG | TTCTCAAAGA | CGATGCTTTC |
| 24751 | GGACGGGGGG | TTTACTTGGA | CCCCCAGTCC | GGCGAGGAGC | TCAACCCAAT |
| | CCTGCCCCCC | AAATGAACCT | GGGGGTCAGG | CCGCTCCTCG | AGTTGGGTTA |
| 24801 | CCCCCGGCCG | CCGCAGCCCT | ATCAGCAGCA | GCCGCGGGCC | CTTGCTTCCC |
| | GGGGGGCGGC | GGCGTCGGGA | TAGTCGTCGT | CGGCGCCCGG | GAACGAAGGG |
| 24851 | AGGATGGCAC | CCAAAAAGAA | GCTGCAGCTG | CCGCCGCCAC | CCACGGACGA |
| | TCCTACCGTG | GGTTTTTCTT | CGACGTCGAC | GGCGGCGGTG | GGTGCTTGCT |
| 24901 | GGAGGAATAC | TGGGACAGTC | AGGCAGAGGA | GGTTTTTGAC | GAGGAGGAGG |
| | CCTCCTTATG | ACCTGTTCAG | TCCGTCTCCT | CCAAAACCTG | CTCCTCCTCC |
| 24951 | AGGACATGAT | GGAAGACTGG | GAGAGCCTAG | ACGAGGAAGC | TTCCGAGGTC |
| | TCCTGTACTA | CCTTCTGACC | CTCTCGGATC | TGCTCCTTCG | AAGGCTCCAG |
| 25001 | GAAGAGGTGT | CAGACGAAAC | ACCGTCACCC | TCGGTCGCAT | TCCCCTCGCC |
| | CTTCTCCACA | GTCTGCTTTG | TGGCAGTGGG | AGCCAGCGTA | AGGGGAGCGG |
| 25051 | GGCGCCCCAG | AAATCGGCAA | CCGGTTCCAG | CATGGCTACA | ACCTCCGCTC |
| | CCGCGGGGTC | TTTAGCCGTT | GGCCAAGGTC | GTACCGATGT | TGGAGGCGAG |
| 25101 | CTCAGGCGCC | GCCGGCACTG | CCCGTTCGCC | GACCCAACCG | TAGATGGGAC |
| | GAGTCCGCGG | CGGCCGTGAC | GGGCAAGCGG | CTGGGTTGGC | ATCTACCCTG |
| 25151 | ACCACTGGAA | CCAGGGCCGG | TAAGTCCAAG | CAGCCGCCGC | CGTTAGCCCA |
| | TGGTGACCTT | GGTCCCGGCC | ATTCAGGTTT | GTCGGCGGCG | GCAATCGGGT |
| 25201 | AGAGCAACAA | CAGCGCCAAG | GCTACCGCTC | ATGGCGCGGG | CACAAGAACG |
| | TCTCGTTGTT | GTCGCGGTTC | CGATGGCGAG | TACCGCGCCC | GTGTTCTTGC |
| 25251 | CCATAGTTGC | TTGCTTGCAA | GACTGTGGGG | GCAACATCTC | CTTCGCCCCG |
| | GGTATCAACG | AACGAACGTT | CTGACACCCC | CGTTGTAGAG | GAAGCGGGCG |
| 25301 | CGCTTTCTTC | TCTACCATCA | CGGCGTGGCC | TTCCCCCGTA | ACATCCTGCA |
| | GCGAAAGAAG | AGATGGTAGT | GCCGCACCGG | AAGGGGGCAT | TGTAGGACGT |
| 25351 | TTACTACCGT | CATCTCTACA | GCCCATACTG | CACCGGCGGC | AGCGGCAGCA |
| | AATGATGGCA | GTAGAGATGT | CGGGTATGAC | GTGGCCGCGG | TCGCCGTCGT |
| 25401 | ACAGCAGCGG | CCACACAGAA | GCAAAGGCGA | CCGGATAGCA | AGACTCTGAC |
| | TGTCGTGCGC | GGTGTGTCTT | CGTTTCCGCT | GGCCTATCGT | TCTGAGACTG |
| 25451 | AAAGCCCAAG | AAATCCACAG | CGGCGGCAGC | AGCAGGAGGA | GGAGCGCTGC |
| | TTTCGGGTTC | TTTAGGTGTC | GCCGCCGTCG | TCGTCTCTCT | CCTCGCGACG |
| 25501 | GTCTGGCGCC | CAACGAACCC | GTATCGACCC | GCGAGCTTAG | AAACAGGATT |
| | CAGACCGCGG | GTTGCTTGGG | CATAGCTGGG | CGCTCGAATC | TTTGTCTTAA |

Figure 27 AA

| | | | | | |
|-------|------------|------------|------------|-------------|------------|
| 25551 | TTTCCCACTC | TGTATGCTAT | ATTTCAACAG | AGCAGGGGCC | AAGAACAAGA |
| | AAAGGGTGAG | ACATACGATA | TAAAGTTGTC | TCGTCCCCGG | TTCTTGTTCT |
| 25601 | GCTGAAAATA | AAAAACAGGT | CTCTGCGATC | CCTCACCCGC | AGCTGCCTGT |
| | CGACTTTTAT | TTTTTGTTCA | GAGACGCTAG | GGAGTGCGG | TCGACGGACA |
| 25651 | ATCACAAAAG | CGAAGATCAG | CTTCGGCGCA | CGCTGGAAGA | CGCGGAGGCT |
| | TAGTGTTTTT | GCTTCTAGTC | GAAGCCGCGT | GCGACCTTCT | GCGCCTCCGA |
| 25701 | CTCTTCAGTA | AATACTGCGC | GCTGACTCTT | AAGGACTAGT | TTGCGGCCCT |
| | GAGAAGTCAT | TTATGACGCG | CGACTGAGAA | TTCCTGATCA | AAGCGCGGGA |
| 25751 | TTCTCAAATT | TAAGCGCGAA | AACTACGTCA | TCTCCAGCGG | CCACACCCGG |
| | AAGAGTTTAA | ATTCGCGCTT | TTGATGCAGT | AGAGGTCGCC | GGTGTGGGCC |
| 25801 | CGCCAGCACC | TGTTGTCAGC | GCCATTATGA | GCAAGGAAAT | TCCACGCCCC |
| | GCGGTCGTGG | ACAACAGTCG | CGGTAATACT | CGTTCCTTTA | AGGGTGCGGG |
| 25851 | TACATGTGGA | GTTACCAGCC | ACAAATGGGA | CTTGCGGCTG | GAGCTGCCCA |
| | ATGTACACCT | CAATGGTCGG | TGTTTACCCT | GAACGCCGAC | CTCGACGGGT |
| 25901 | AGACTACTCA | ACCCGAATAA | ACTACATGAG | CGCGGGACCC | CACATGATAT |
| | TCTGATGAGT | TGGGCTTATT | TGATGTACTC | GCGCCCTGGG | GTGTACTATA |
| 25951 | CCCGGGTCAA | CGGAATACGC | GCCCACCGAA | ACCGAATTCT | CCTGGAACAG |
| | GGGCCAGTT | GCCTTATGCG | CGGGTGCGTT | TGGCTTAAGA | GGACCTTGTC |
| 26001 | GCGGCTATTA | CCACCACACC | TCGTAATAAC | CTTAATCCCC | GTAGTTGGCC |
| | CGCCGATAAT | GGTGGTGTGG | AGCATTATTG | GAATTAGGGG | CATCAACCGG |
| 26051 | CGTGCCCTG | GTGTACCAGG | AAAGTCCCGC | TCCCACCACT | GTGGTACTTC |
| | GCGACGGGAC | CACATGGTCC | TTTCAGGGCG | AGGGTGGTGA | CACCATGAAG |
| 26101 | CCAGAGACGC | CCAGGCCGAA | GTTCAGATGA | CTAACTCAGG | GGCGCAGCTT |
| | GGTCTCTGCG | GGTCCGGCTT | CAAGTCTACT | GATTGAGTCC | CCGCGTCGAA |
| 26151 | GCGGGCGGCT | TTCGTACACG | GGTGCGGTCG | CCCGGGCAGG | GTATAACTCA |
| | CGCCCGCCGA | AAGCAGTGTC | CCACGCCAGC | GGGCCCCTCC | CATATTGAGT |
| 26201 | CCTGACAATC | AGAGGGCGAG | GTATTGAGCT | CAACGACGAG | TCGGTGAGCT |
| | GGACTGTTAG | TCTCCCGCTC | CATAAGTCGA | GTTGCTGCTC | AGCCACTCGA |
| 26251 | CCTCGCTTGG | TCTCCGTCCG | GACGGGACAT | TTCAGATCGG | CGGCGCCGGC |
| | GGAGCGAACC | AGAGGCAGGC | CTGCCCTGTA | AAGTCTAGCC | GCCGCGGCCG |
| 26301 | CGCTCTTCAT | TCACGCCTCG | TCAGGCAATC | CTAACTCTGC | AGACCTCGTC |
| | GCGAGAAGTA | AGTGCGGAGC | AGTCCGTTAG | GATTGAGACG | TCTGGAGCAG |
| 26351 | CTCTGAGCCG | CGCTCTGGAG | GCATTGGAAC | TCTGCAATTT | ATTGAGGAGT |
| | GAGACTCGGC | GCGAGACCTC | CGTAACCTTG | AGACGTTAAA | TAACTCCTCA |
| 26401 | TTGTGCCATC | GGTCTACTTT | AACCCCTTCT | CGGGACCTCC | CGGCCACTAT |
| | AACACGGTAG | CCAGATGAAA | TTGGGGAAGA | GCCCTGGAGG | GCCGGTGATA |
| 26451 | CCGGATCAAT | TTATTCTTAA | CTTTGACGCG | GTAAAGGACT | CGGCGGACGG |
| | GGCCTAGTTA | AATAAGGATT | GAAACTGCGC | CATTTCTCTGA | GCCGCTGCCC |

Figure 27 AB

| | | | | | |
|-------|------------|-------------|-------------|-------------|------------|
| 26501 | CTACGACTGA | ATGTTAAGTG | GAGAGGCAGA | GCAACTGCGC | CTGAAACACC |
| | GATGCTGACT | TACAATTCAC | CTCTCCGTCT | CGTTGACGCG | GACTTTGTGG |
| 26551 | TGGTCCACTG | TCGCCGCCAC | AAGTGCTTTG | CCCGCGACTC | CGGTGAGTTT |
| | ACCAGGTGAC | AGCGGCGGTG | TTACGAAAC | GGGCGCTGAG | GCCACTCAAA |
| 26601 | TGCTACTTTG | AATTGCCCCG | GGATCATATC | GAGGGCCCCG | CGCACGGCGT |
| | ACGATGAAAC | TTAACGGGCT | CCTAGTATAG | CTCCCGGGCC | GCGTGCCGCA |
| 26651 | CCGGCTTACC | GCCCAGGGAG | AGCTTGCCCC | TAGCCTGATT | CGGGAGTTTA |
| | GGCCGAATGG | CGGGTCCCTC | TCGAACGGGC | ATCGGACTAA | GCCCTCAAAT |
| 26701 | CCCAGCGCCC | CCTGCTAGTT | GAGCGGGACA | GGGGACCCCTG | TGTTCTCACT |
| | GGGTGCGGGG | GGACGATCAA | CTCGCCCTGT | CCCCTGCGGAC | ACAAGAGTGA |
| 26751 | GTGATTTGCA | ACTGTCCTAA | CCCTGGATTA | CATCAAGATC | TTTGTTGCCA |
| | CACTAAACGT | TGACAGGATT | GGGACCTAAT | GTAGTTCTAG | AAACAACGGT |
| 26801 | TCTCTGTGCT | GAGTATAATA | AATACAGAAA | TTAAAAATATA | CTGGGGCTCC |
| | AGAGACACGA | CTCATATTAT | TTATGTCTTT | AATTTTATAT | GACCCCGAGG |
| 26851 | TATCGCCATC | CTGTAAACGC | CACCGTCTTC | ACCCGCCCAA | GCAAACCAAG |
| | ATAGCGGTAG | GACATTTGCG | GTGGCAGAAG | TGGGCGGGTT | CGTTTGTTTC |
| 26901 | GCGAACCTTA | CCTGGTACTT | TTAACATCTC | TCCCTCTGTG | ATTTACAACA |
| | CGCTTGGAAT | GGACCATGAA | AATTGTAGAG | AGGGAGACAC | TAAATGTTGT |
| 26951 | GTTTCAACCC | AGACGGAGTG | AGTCTACGAG | AGAACCCTCTC | CGAGCTCAGC |
| | CAAAGTTGGG | TCTGCCTCAC | TCAGATGCTC | TCTTGAGAG | GCTCGAGTCG |
| 27001 | TACTCCATCA | GAAAAACAC | CACCCCTCCTT | ACCTGCCGGG | AACGTACGAG |
| | ATGAGGTAGT | CTTTTTTGTTG | GTGGGAGGAA | TGGACGGCCC | TTGCATGCTC |
| 27051 | TGCGTCACCG | GCCGCTGCAC | CACACCTACC | GCCTGACCGT | AAACCAGACT |
| | ACGCAGTGGC | CGGCGACGTG | GTGTGGATGG | CGGACTGGCA | TTTGGTCTGA |
| 27101 | TTTTCCGGAC | AGACCTCAAT | AACCTCTGTTT | ACCAGAACAG | GAGGTGAGCT |
| | AAAAGGCCTG | TCTGGAGTTA | TTGAGACAAA | TGGTCTTGTC | CTCCACTCGA |
| 27151 | TAGAAAACCC | TTAGGGTATT | AGGCCAAAGG | CGCAGCTACT | GTGGGGTTTA |
| | ATCTTTTGGG | AATCCCATAA | TCCGGTTTCC | GCGTCGATGA | CACCCCAAAT |
| 27201 | TGAACAATTC | AAGCAACTCT | ACGGGCTATT | CTAATTCAGG | TTTCTCTAGA |
| | ACTTGTTAAG | TTCGTTGAGA | TGCCCGATAA | GATTAAAGTCC | AAAGAGATCT |
| 27251 | ATCGGGGTTG | GGGTTATTCT | CTGTCTTTGTG | ATTCTCTTTA | TTCTTATACT |
| | TAGCCCCAAC | CCCAATAAGA | GACAGAACAC | TAAGAGAAAT | AAGAATATGA |
| 27301 | AACGCTTCTC | TGCCTAAGGC | TCGCCGCCCTG | CTGTGTGCAC | ATTTGCATTT |
| | TTGCCAAGAG | ACGGATTCCG | AGCGGCGGAC | GACACACGTG | TAAACGTAA |
| 27351 | ATTGTCAGCT | TTTTAAACGC | TGGGGTCGCC | ACCCAAGATG | ATTAGGTACA |
| | TAACAGTCGA | AAAATTTGCG | ACCCAGCGG | TGGGTTCTAC | TAATCCATGT |
| 27401 | TAATCCTAGG | TTTACTCACC | CTTGCGTCAG | CCCACGGTAC | CACCCAAAAG |
| | ATTAGGATCC | AAATGAGTGG | GAACGCAGTC | GGGTGCCATG | GTGGGTTTTT |

Figure 27AC

27451 GTGGATTTTA AGGAGCCAGC CTGTAATGTT ACATTTCGCAG CTGAAAGCTAA
CACCTAAAAT TCCTCGGTCG GACATTACAA TGTAAGCGTC GACTTCGATT

27501 TGAGTGCACC ACTCTTATAA AATGCACCAC AGAACATGAA AAGCTGCTTA
ACTCACGTGG TGAGAATATT TTACGTGGTG TCTTGTAATT TTCGACGAAT

27551 TTCGCCACAA AAACAAAATT GGCAAGTATG CTGTTTATGC TATTTGGCAG
AAGCGGTGTT TTTGTTTAA CCGTTCATAC GACAAATACG ATAAACCGTC

27601 CCAGGTGACA CTACAGAGTA TAATGTTACA GTTTTCCAGG GTAAAGTCA
GGTCCACTGT GATGTCTCAT ATTACAATGT CAAAAGGTCC CATTTTCAGT

27651 TAAAACTTTT ATGTATACTT TTCCATTTTA TGAAATGTGC GACATTACCA
ATTTTGAAAA TACATATGAA AAGGTAAAAT ACTTTACACG CTGTAATGGT

27701 TGTACATGAG CAAACAGTAT AAGTTGTGGC CCCACAAAA TTGTGTGGAA
ACATGTACTC GTTTGTCATA TTCAACACCG GGGGTGTTTT AACACACCTT

27751 AACACTGGCA CTTTCTGCTG CACTGCTATG CTAATTACAG TGCTCGCTTT
TTGTGACCGT GAAAGACGAC GTGACGATAC GATTAATGTC ACGAGCGAAA

27801 GGTCTGTACC CTACTCTATA TTAAATACAA AAGCAGACGC AGCTTTATTG
CCAGACATGG GATGAGATAT AATTTATGTT TTCGTCTGCG TCGAAATAAC

27851 AGGAAAAGAA AATGCCTTAA TTTACTAAGT TACAAAGCTA ATGTCACCAC
TCCTTTTCTT TTACGGAATT AAATGATTCA ATGTTTCGAT TACAGTGGTG

27901 TAACTGCTTT ACTCGCTGCT TGCAAAACAA ATTCAAAAAG TTAGCATTAT
ATTGACGAAA TGAGCGACGA ACGTTTTGTT TAAGTTTTTC AATCGTAATA

27951 AATTAGAATA GGATTTAAAC CCCCCGGTCA TTTCTGCTC AATACCATT
TTAATCTTAT CCTAAATTG GGGGGCCAGT AAAGGACGAG TTATGGTAAG

28001 CCCTGAACAA TTGACTCTAT GTGGGATATG CTCCAGCGCT ACAACCTTGA
GGGACTTGTT AACTGAGATA CACCCTATAC GAGGTCGCGA TGTTGGAAT

28051 AGTCAGGCTT CCTGGATGTC AGCATCTGAC TTTGGCCAGC ACCTGTCCCG
TCAGTCCGAA GGACCTACAG TCGTAGACTG AAACCGGTCG TGGACAGGGC

28101 CGGATTTGTT CCAGTCCAAC TACAGCGACC CACCCTAACA GAGATGACCA
GCCTAAACAA GGTGAGGTTG ATGTCGCTGG GTGGGATTGT CTCTACTGGT

28151 ACACAACCAA CGCGGCCGCC GCTACCGGAC TTACATCTAC CACAAATACA
TGTGTTGGTT GCGCCGGCGG CGATGGCCTG AATGTAGATG GTGTTTATGT

28201 CCCCAGTTT CTGCCTTTGT CAATAACTGG GATAACTTGG GCATGTGGTG
GGGGTTCAAA GACGGAAACA GTTATTGACC CTATTGAACC CGTACACCAC

28251 GTTCTCCATA GCGCTTATGT TTGTATGCCT TATTATTATG TGGCTCATCT
CAAGAGGTAT CGCGAATACA AACATACGGA ATAATAATAC ACCGAGTAGA

28301 GCTGCCTAAA GCGCAAACGC GCCCAGCAC CCATCTATAG TCCCATCATT
CGACGGATTT CGCGTTTGCG CGGGCTGGTG GGTAGATATC AGGGTAGTAA

28351 GTGCTACACC CAAACAATGA TGGAATCCAT AGATTGGACG GACTGAAACA
CACGATGTGG GTTTGTTACT ACCTTAGGTA TCTAACCTGC CTGACTTTGT

Figure 27A D

| | | | | | |
|-------|------------|------------|------------|------------|-------------|
| 28451 | TTTTATATTA | CTGACCCTTG | TTGCGCTTTT | TTGTGCGTGC | TCCACATTGG |
| | AAAATATAAT | GACTGGGAAC | AACGCGAAAA | AACACGCACG | AGGTGTAACC |
| 28501 | CTGCGGTTTC | TCACATCGAA | GTAGACTGCA | TTCCAGCCTT | CACAGTCTAT |
| | GACGCCAAAG | AGTGTAGCTT | CATCTGACGT | AAGGTCGGAA | GTGTCAGATA |
| 28551 | TTGCTTTACG | GATTTGTCAC | CCTCACGCTC | ATCTGCAGCC | TCATCACTGT |
| | AACGAAATGC | CTAAACAGTG | GGAGTGCAG | TAGACGTCGG | AGTAGTGACA |
| 28601 | GGTCATCGCC | TTTATCCAGT | GCATTGACTG | GGTCTGTGTG | CGCTTTGTCAT |
| | CCAGTAGCGG | AAATAGGTCA | CGTAACTGAC | CCAGACACAC | GCGAAACGTA |
| 28651 | ATCTCAGACA | CCATCCCCAG | TACAGGGACA | GGACTATAGC | TGAGCTTCTT |
| | TAGAGTCTGT | GGTAGGGGTC | ATGTCCCTGT | CCTGATATCG | ACTCGAAGAA |
| 28701 | AGAATTCTTT | AATTATGAAA | TTACTGTGA | CTTTTCTGCT | GATTATTTGC |
| | TCTTAAGAAA | TTAATACTTT | AAATGACACT | GAAAAGACGA | CTAATAAACG |
| 28751 | ACCCTATCTG | CGTTTTGTTC | CCCGACCTCC | AAGCCTCAAA | GACATATATC |
| | TGGGATAGAC | GCAAAACAAG | GGGCTGGAGG | TTCGGAGTTT | CTGTATATAG |
| 28801 | ATGCAGATTC | ACTCGTATAT | GGAATATTCC | AAGTTGCTAC | AATGAAAAAA |
| | TACGTCTAAG | TGAGCATATA | CCTTATAAGG | TTCAACGATG | TTACTTTTTT |
| 28851 | GCGATCTTTC | CGAAGCCTGG | TTATATGCAA | TCATCTCTGT | TATGGTGTTC |
| | CGCTAGAAAG | GCTTCGGACC | AATATACGTT | AGTAGAGACA | ATACCACAAG |
| 28901 | TGCAGTACCA | TCTTAGCCCT | AGCTATATAT | CCCTACCTTG | ACATTGGCTG |
| | ACGTCATGGT | AGAATCGGGA | TCGATATATA | GGGATGGAAC | TGTAACCGAC |
| 28951 | GAACGCAATA | GATGCCATGA | ACCACCCAAC | TTTCCCCGCG | CCCGCTATGC |
| | CTTGCGTTAT | CTACGGTACT | TGGTGGGTTG | AAAGGGGCGC | GGGCGATACG |
| 29001 | TTCCACTGCA | ACAAGTTGTT | GCCGGCGGCT | TTGTCCCAGC | CAATCAGCCT |
| | AAGGTGACGT | TGTTCAACAA | CGGCCGCCGA | AACAGGGTCG | GTTAGTCGGA |
| 29051 | CGCCACCTT | CTCCACCCC | CACTGAAATC | AGCTACTTTA | ATCTAACAGG |
| | GCGGGTGGAA | GAGGGTGGGG | GTGACTTTAG | TCGATGAAAT | TAGATTGTCC |
| 29101 | AGGAGATGAC | TGACACCCTA | GATCTAGAAA | TGGACGGAAT | TATTACAGAG |
| | TCCTCTACTG | ACTGTGGGAT | CTAGATCTTT | ACCTGCCTTA | ATAATGTCTC |
| 29151 | CAGCGCCTGC | TAGAAAGACG | CAGGGCAGCG | GCCGAGCAAC | AGCGCATGAA |
| | GTGCGGGACG | ATCTTTCTGC | GTCCCGTCGC | CGGCTCGTTG | TCGCGTACTT |
| 29201 | TCAAGAGCTC | CAAGACATGG | TTAACTTGCA | CCAGTGCAAA | AGGGGTATCT |
| | AGTTCTCGAG | GTTCTGTACC | AATTGAACGT | GGTCACGTTT | TCCCCATAGA |
| 29251 | TTGTCTCGT | AAAGCAGGCC | AAAGTCACCT | ACGACAGTAA | TACCACCGGA |
| | AAACAGAGCA | TTTCGTCCGG | TTTCAGTGGA | TGCTGTCATT | ATGGTGGCCT |
| 29301 | CACCGCCTTA | GCTACAAGTT | GCCAACCAAG | CGTCAGAAAT | TGGTGGTCAT |
| | GTGGCGGAAT | CGATGTTCAA | CGGTTGGTTC | GCAGTCTTTA | ACCACCAGTA |

Figure 27 A E

29401 GCTGCATTCA CTCACCTTGT CAAGGACCTG AGGATCTCTG CACCCTTATT
CGACGTAAGT GAGTGGAAAC GTTCCTGGAC TCCTAGAGAC GTGGGAATAA

29451 AAGACCTTGT GCGGTCTCAA AGATCTTATT CCCTTTAACT AATAAAAAAA
TTCTGGGACA CGCCAGAGTT TCTAGAATAA GGGAAATTGA TTATTTTATT

29501 AATAATAAAG CATCACTTAC TTAAAATCAG TTAGCAAATT TCTGTCCAGT
TTATTATTTC GTAGTGAATG AATTTTAGTC AATCGTTTAA AGACAGGTCA

29551 TTATTCAGCA GCACCTCCTT GCCCTCCTCC CAGCTCTGGT ATTGCAGCTT
AATAAGTCGT CGTGGAGGAA CGGGAGGAGG GTCGAGACCA TAACGTCGAA

29601 CCTCCTGGCT GCAAACCTTC TCCACAATCT AAATGGAATG TCAGTTTCCT
GGAGGACCGA CGTTTGAAAG AGGTGTTAGA TTTACCTTAC AGTCAAAGGA

29651 CCTGTTCCCTG TCCATCCGCA CCCACTATCT TCATGTTGTT GCAGATGAAG
GGACAAGGAC AGGTAGGCGT GGGTGATAGA AGTACAACAA CGTCTACTTC

29701 CGCGCAAGAC CGTCTGAAGA TACCTTCAAC CCCGTGTATC CATATGACAC
GCGCGTTCTG GCAGACTTCT ATGGAAGTTG GGGCACATAG GTATACTGTG

29751 GGAAACCGGT CCTCCAACCTG TGCCTTTTCT TACTCCTCCC TTTGTATCCC
CCTTTGGCCA GGAGGTTGAC ACGGAAAAGA ATGAGGAGGG AAACATAGGG

29801 CCAATGGGTT TCAAGAGAGT CCCCTGGGG TACTCTCTTT GCGCCTATCC
GGTTACCCAA AGTTCTCTCA GGGGGACCCC ATGAGAGAAA CGCGGATAGG

29851 GAACCTCTAG TTACCTCCAA TGGCATGCTT GCGCTCAAAA TGGGCAACGG
CTTGGAGATC AATGGAGGTT ACCGTACGAA CGCGAGTTTT ACCCGTTGCC

29901 CCTCTCTCTG GACGAGGCCG GCAACCTTAC CTCCCAAAT GTAACCACTG
GGAGAGAGAC CTGCTCCGGC CGTTGGAATG GAGGGTTTTA CATTGGTGAC

29951 TGAGCCCACC TCTCAAAAAA ACCAAGTCAA ACATAAACCT GGAAATATCT
ACTCGGGTGG AGAGTTTTTT TGGTTCAGTT TGTATTTGGA CCTTTATAGA

30001 GCACCCCTCA CAGTTACCTC AGAAGCCCTA ACTGTGGCTG CCGCCGCACC
CGTGGGGAGT GTCAATGGAG TCTTCGGGAT TGACACCGAC GCGGGCGTGG

30051 TCTAATGGTC GCGGGCAACA CACTCACCAT GCAATCACAG GCCCCGCTAA
AGATTACCAG CGCCCGTTGT GTGAGTGGTA CGTTAGTGTC CGGGGCGATT

30101 CCGTGACGCA CTCCAAACTT AGCATTGCCA CCCAAGGACC CCTCACAGTG
GGCACGTGCT GAGGTTTGAA TCGTAACGGT GGGTTCCTGG GGAGTGTAC

30151 TCAGAAGGAA AGCTAGCCCT GCAAACATCA GGCCCCCTCA CCACCACCGA
AGTCTTCCTT TCGATCGGGA CGTTGTAGT CCGGGGGAGT GGTGGTGGCT

30201 TAGCAGTACC CTTACTATCA CTGCCTCACC CCCTCTAACT ACTGCCACTG
ATCGTCATGG GAATGATAGT GACGGAGTGG GGGAGATTGA TGACGGTGAC

30251 GTAGCTTGGG CATTGACTTG AAAGAGCCCA TTTATACACA AAATGGAAAA
CATCGAACCC GTAACCTGAAC TTTCTCGGGT AAATATGTGT TTTACCTTTT

Figure 27 AF

30351 TTTGACCGTA GCAACTGGTC CAGGTGTGAC TATTAATAAT ACTTCCTTGC
AAACTGGCAT CGTTGACCAG GTCCACACTG ATAATTATTA TGAAGGAACG

30401 AAACATAAGT TACTGGAGCC TTGGGTTTTG ATTCACAAGG CAATATGCAA
TTTGATTTCA ATGACCTCGG AACCCAAAAC TAAGTGTTCG GTTATACGTT

30451 CTTAATGTAG CAGGAGGACT AAGGATTGAT TCTCAAAACA GACGCCTTAT
GAATTACATC GTCCCTCCTGA TTCCTAACTA AGAGTTTTGT CTGCGGAATA

30501 ACTTGATGTT AGTTATCCGT TTGATGCTCA AAACCAACTA AATCTAAGAC
TGAACATACA TCAATAGGCA AACTACGAGT TTTGGTTGAT TTAGATTCTG

30551 TAGGACAGGG CCTCTTTTTT ATAACTCAG CCCACAACCTT GGATATTAAC
ATCCTGTCCC GGGAGAAAAA TATTTGAGTC GGGTGTGAA CCTATAATTG

30601 TACAACAAAG GCCTTTACTT GTTTACAGCT TCAAACAATT CCAAAAAGCT
ATGTTGTTTC CGGAAATGAA CAAATGTCGA AGTTTGTAA GGTTTTTTCGA

30651 TGAGGTAAAC CTAAGCACTG CCAAGGGGTT GATGTTTGAC GCTACAGCCA
ACTCCAATTG GATTCGTGAC GGTCCCCAA CTACAACTG CGATGTCGGT

30701 TAGCCATTAA TGCAGGAGAT GGGCTTGAAT TTGGTTCACC TAATGCACCA
ATCGGTAATT ACGTCCTCTA CCCGAACTTA AACCAGTGG ATTACGTGGT

30751 AACACAAATC CCTCAAAAC AAAAATTGGC CATGGCCTAG AATTTGATTC
TTGTGTTTAG GGGAGTTTTG TTTTAAACCG GTACCGGATC TTAAACTAAG

30801 AAACAAGGCT ATGGTTCCTA AACTAGGAAC TGGCCTTAGT TTTGACAGCA
TTTGTTCCGA TACCAAGGAT TTGATCCTTG ACCGGAATCA AAACGTGCTG

30851 CAGGTGCCAT TACAGTAGGA AACAAAAATA ATGATAAGCT AACTTTGTGG
GTCCACGGTA ATGTCATCCT TTGTTTTTAT TACTATTCGA TTGAAACACC

30901 ACCACACCAG CTCCATCTCC TAACTGTAGA CTAAATGCAG AGAAAGATGC
TGGTGTGGTC GAGGTAGAGG ATTGACATCT GATTTACGTC TCTTCTACG

30951 TAAACTCACT TTGGTCTTAA CAAAATGTGG CAGTCAAATA CTTGCTACAG
ATTTGAGTGA AACCAGAATT GTTTTACACC GTCAGTTTAT GAACGATGTC

31001 TTTCAGTTTT GGCTGTTAAA GGCAGTTTGG CTCCAATATC TGGAACAGTT
AAAGTCAAAA CCGACAATTT CCGTCAAACC GAGGTTATAG ACCTTGTCAA

31051 CAAAGTGCTC ATCTTATTAT AAGATTTGAC GAAAATGGAG TGCTACTAAA
GTTTCACGAG TAGAATAATA TTCTAAACTG CTTTACCTC ACGATGATTT

31101 CAATTCCCTC CTGGACCCAG AATATTGGAA CTTTAGAAAT GGAGATCTTA
GTTAAGGAAG GACCTGGGTC TTATAACCTT GAAATCTTTA CCTCTAGAAT

31151 CTGAAGGCAC AGCCTATACA AACGCTGTTG GATTTATGCC TAACCTATCA
GACTTCCGTG TCGGATATGT TTGCGACAAC CTAAATACGG ATTGGATAGT

31201 GCTTATCCAA AATCTCACGG TAAACTGCC AAAAGTAACA TTGTCAGTCA
CGAATAGGTT TTAGAGTGCC ATTTTGACGG TTTTCATTGT AACAGTCAGT

Figure 27 AG

31251 AGTTTACTTA AACGGAGACA AAATAAACC TGTAACACTA ACCATTACAC
 TCAAATGAAT TTGCCTCTGT TTTGATTGG ACATTGTGAT TGGTAATGTG

31301 TAAACGGTAC ACAGGAAACA GGAGACACAA CTCCAAGTGC ATACTCTATG
 ATTTGCCATG TGTCTTTGT CCTCTGTGTT GAGGTTACAG TATGAGATAC

31351 TCATTTTCAT GGGACTGGTC TGGCCACAAC TACATTAATG AAATATTTGC
 AGTAAAAGTA CCCTGACCAG ACCGGTGTG ATGTAATTAC TTTATAAACG

31401 CACATCCTCT TACACTTTTT CATAATTGC CCAAGAATAA AGAATCGTTT
 GTGTAGGAGA ATGTGAAAAA GTATGTAACG GGTTCCTATT TCTTAGCAAA

31451 GTGTTATGTT TCAACGTGTT TATTTTTCAA TTGCAGAAAA TTTCAAGTCA
 CACAATACAA AGTTGCACAA ATAAAAAGTT AACGTCCTTT AAAGTTCAGT

31501 TTTTTCATT CAGTAGTATAG CCCCACCACC ACATAGCTTA TACAGATCAC
 AAAAAGTAAG TCATCATATC GGGGTGGTGG TGTATCGAAT ATGTCTAGTG

31551 CGTACCTTAA TCAAACTCAC AGAACCCCTAG TATTCAACCT GCCACCTCCC
 GCATGGAATT AGTTTGAGTG TCTTGGGATC ATAAGTTGGA CGGTGGAGGG

31601 TCCCAACACA CAGAGTACAC AGTCCTTTCT CCCC GGCTGG CCTTAAAAAG
 AGGGTTGTGT GTCTCATGTG TCAGGAAAGA GGGGCCGACC GGAATTTTTC

31651 CATCATATCA TGGGTAACAG ACATATTCTT AGGTGTTATA TTCCACACGG
 GTAGTATAGT ACCCATTGTC TGTATAAGAA TCCACAATAT AAGGTGTGCC

31701 TTTCTGTGCG AGCCAAACGC TCATCAGTGA TATTAATAAA CTCCCCGGGC
 AAAGGACAGC TCGGTTTGCG AGTAGTCACT ATAATTATTT GAGGGGCCCG

31751 AGCTCACTTA AGTTCATGTC GCTGTCCAGC TGCTGAGCCA CAGGCTGCTG
 TCGAGTGAAT TCAAGTACAG CGACAGGTCG ACGACTCGGT GTCCGACGAC

31801 TCCAACTTGC GGTGCTTAA CGGGCGGCGA AGGAGAAGTC CACGCCTACA
 AGGTGTAACG CCAACGAATT GCGCGCCGCT TCCTCTTCAG GTGCGGATGT

31851 TGGGGGTAGA GTCATAATCG TGCATCAGGA TAGGGCGGTG GTGCTGCAGC
 ACCCCCATCT CAGTATTAGC ACGTAGTCCT ATCCCGCCAC CACGACGTG

31901 AGCGCGCGAA TAACTGCTG CCGCCGCCGC TCCGTCTG CAGGAATACAA
 TCGCGCGCTT ATTTGACGAC GGCGCGGCG AGGCAGGACG TCCTTATGTT

31951 CATGGCAGTG GTCTCTCAG CGATGATTG CACCGCCCGC AGCATAAGGC
 GTACCGTCAC CAGAGGAGTC GCTACTAAGC GTGGCGGGCG TCGTATTCCG

32001 GCCTTGTCTT CCGGGCACAG CAGCGCACC TGATCTCACT TAAATCAGCA
 CGGAACAGGA GGCCCGTGTG GTGCGTGGG ACTAGAGTGA ATTTAGTCGT

32051 CAGTAACTGC AGCACAGCAC CACAATATTG TTCAAATCC CACAGTGCAA
 GTCATTGACG TCGTGTCTGT GTGTTATAAC AAGTTTTAGG GTGTCACGTT

32101 GGCGCTGTAT CCAAAGCTCA TGGCGGGGAC CACAGAACCC ACGTGGCCAT
 CCGCGACATA GGTTCGAGT ACCGCCCTG GTGTCCTGGG TGCACCGGTA

32151 CATACCACAA GCGCAGGTAG ATTAAGTGGC GACCCCTCAT AAACACGCTG
 GTATGGTGTG CGCGTCCATC TAATTCACCG CTGGGGAGTA TTTGTGCGAC

Figure 27AH

32251 CCATATAAAC CTCTGATTAA ACATGGCGCC ATCCACCACC ATCCTAAACC
 GGTATATTTG GAGACTAATT TGTACCGCGG TAGGTGGTGG TAGGATTTGG

 32301 AGCTGGCCAA AACCTGCCCC CCGGCTATAC ACTGCAGGGA ACCGGGACTG
 TCGACCGGTT TTGGACGGGC GGCCGATATG TGACGTCCCT TGGCCCTGAC

 32351 GAACAATGAC AGTGGAGAGC CCAGGACTCG TAACCATGGA TCATCATGCT
 CTTGTTACTG TCACCTCTCG GGTCTTGAGC ATTGGTACCT AGTAGTACGA

 32401 CGTCATGATA TCAATGTTGG CACAACACAG GCACACGTGC ATACACTTCC
 GCAGTACTAT AGTTACAACC GTGTTGTGTC CGTGTGCACG TATGTGAAGG

 32451 TCAGGATTAC AAGCTCCTCC CGCGTTAGAA CCATATCCCA GGAACAACC
 AGTCCTAATG TTCGAGGAGG GCGCAATCTT GGTATAGGGT CCTTGTGTGG

 32501 CATTCCTGAA TCAGCGTAAA TCCCACACTG CAGGGAAGAC CTCGCACGTA
 GTAAGGACTT AGTCGCATTT AGGGTGTGAC GTCCCTTCTG GAGCGTGCAT

 32551 ACTCACGTTG TGCATTGTCA AAGTGTTACA TTCGGGCAGC AGCGGATGAT
 TGAGTGCAAC ACGTAACAGT TTCACAATGT AAGCCCGTCG TCGCCTACTA

 32601 CCTCCAGTAT GGTAGCGCGG GTTCTGTCT CAAAAGGAGG TAGACGATCC
 GGAGGTCATA CCATCGCGCC CAAAGACAGA GTTTTCCTCC ATCTGCTAGG

 32651 CTA CTGTACG GAGTGCGCC AGACAACCGA GATCGTGTTG GTCGTAGTGT
 GATGACATGC CTCACGCGGC TCTGTTGGCT CTAGCACAA CAGCATCACA

 32701 CATGCCAAAT GGAACGCCCG ACGTAGTCAT ATTTCTGAA GCAAAACCAG
 GTACGGTTTA CCTTGCGGCC TGCATCAGTA TAAAGGACTT CGTTTTGGTC

 32751 GTGCGGGCGT GACAAACAGA TCTGCGTCTC CGGTCTCGCC GCTTAGATCG
 CACGCCCGCA CTGTTTGTCT AGACGCAGAG GCCAGAGCGG CGAATCTAGC

 32801 CTCTGTGTAG TAGTTGTAGT ATATCCACTC TCTCAAAGCA TCCAGGCGCC
 GAGACACATC ATCAACATCA TATAGGTGAG AGAGTTTCGT AGGTCCGCGG

 32851 CCCTGGCTTC GGGTTCTATG TAAACTCCTT CATGCGCCGC TGCCCTGATA
 GGGACCGAAG CCCAAGATAC ATTTGAGGAA GTACGCGGCG ACGGGACTAT

 32901 ACATCCACCA CCGCAGAATA AGCCACACCC AGCCAACCTA CACATTCGTT
 TGTAGGTGGT GCGCTCTTAT TCGGTGTGGG TCGGTGGAT GTGTAAGCAA

 32951 CTGCGAGTCA CACACGGGAG GAGCGGGAAG AGCTGGAAGA ACCATGTTTT
 GACGCTCAGT GTGTGCCCTC CTCGCCCTTC TCGACCTTCT TGGTACAAAA

 33001 TTTTTTTTATT CAAAAGATT ATCCAAAACC TCAAAATGAA GATCTATTAA
 AAAAAATAA GGTTTTCTAA TAGGTTTTGG AGTTTTACTT CTAGATAATT

 33051 GTGAACGCGC TCCCCTCGG TGGCGTGGTC AAACCTCTACA GCCAAAGAAC
 CACTTGCGCG AGGGGAGGCC ACCGCACCAG TTTGAGATGT CGGTTCTTTC

 33101 AGATAATGGC ATTTGTAAGA TGTGTCACAA TGGCTTCCAA AAGGCAAACG
 TCTATTACCG TAAACATTCT ACAACGTGTT ACCGAAGGTT TTCCGTTTGC

Figure 27 AI

33201 CTCTATAAAC ATTCCAGCAC CTTCAACCAT GCCCAAATAA TTCTCATCTC
 GAGATATTTG TAAGGTCGTG GAAGTTGGTA CGGGTTTATT AAGAGTAGAG
 33251 GCCACCTTCT CAATATATCT CTAAGCAAAT CCCGAATATT AAGTCCGGCC
 CGGTGGAAGA GTTATATAGA GATTCTGTTA GGGCTTATAA TTCAGGCCCG
 33301 ATTGTAAAAA TCTGCTCCAG AGCGCCCTCC ACCTTCAGCC TCAAGCAGCG
 TAACATTTTT AGACGAGGTC TCGCGGGAGG TGAAGTCGG AGTTCGTCTC
 33351 AATCATGATT GCAAAAATTC AGGTTCTCTA CAGACCTGTA TAAGATTCAA
 TTAGTACTAA CGTTTTTAAG TCCAAGGAGT GTCTGGACAT ATTCTAAGTT
 33401 AAGCGGAACA TTAACAAAAA TACCGCGATC CCGTAGGTCC CTTTCGAGGG
 TTCGCCCTTGT AATTGTTTTT ATGGCGCTAG GGCATCCAGG GAAGCGTCCC
 33451 CCAGCTGAAC ATAATCGTGC AGGTCTGCAC GGACCAGCGC GGCCACTTCC
 GGTCGACTTG TATTAGCACG TCCAGACGTG CCTGGTCGCG CCGGTGAAGG
 33501 CCGCCAGGAA CCATGACAAA AGAACCACCA CTGATTATGA CACGCATACT
 GGCGGTCCCTT GGTACTGTTT TCTTGGGTGT GACTAATACT GTGCGTATGA
 33551 CGGAGCTATG CTAACCAGCG TAGCCCCGAT GTAAGCTTGT TGCATGGGCG
 GCCTCGATAC GATTGGTCGC ATCGGGGCTA CATTCCAACA ACGTACCCGC
 33601 GCGATATAAA ATGCAAGGTG CTGCTCAAAA AATCAGGCAA AGCCTCGCGC
 CGCTATATTT TACGTTCAC GACGAGTTTT TTAGTCCGTT TCGGAGCGCG
 33651 AAAAAAGAAA GCACATCGTA GTCATGCTCA TGCAGATAAA GGCAGGTAAG
 TTTTTCTTT CGTGTAGCAT CAGTACGAGT ACGTCTATTT CCGTCCATTC
 33701 CTCCGGAACC ACCACAGAAA AAGACACCAT TTTTCTCTCA AACATGTCTG
 GAGGCCCTGG TGGTGTCTTT TTCTGTGGTA AAAAGAGAGT TTGTACAGAC
 33751 CGGGTTTCTG CATAAACACA AAATAAAATA ACAAAAAAAC ATTTAAACAT
 GCCCAAAGAC GTATTTGTGT TTTATTTTAT TGTTTTTTTG TAAATTTGTA
 33801 TAGAAGCCTG TCTTACAACA GGAAAAACAA CCCTTATAAG CATAAGACGG
 ATCTTCGGAC AGAATGTTGT CCTTTTGTGTT GGGAATATTC GTATTCTGCC
 33851 ACTACGGCCA TGCCGGCGTG ACCGTAAAAA AACTGGTCAC CGTGATTAAA
 TGATGCCGGT ACGGCCGCAC TGGCATTTTT TTGACCAGTG GCACTAATTT
 33901 AAGCACCACC GACAGCTCCT CGGTCATGTC CGGAGTCATA ATGTAAGACT
 TTCGTGGTGG CTGTCGAGGA GCCAGTACAG GCCTCAGTAT TACATTCTGA
 33951 CGGTAAACAC ATCAGGTTGA TTCACATCGG TCAGTGCTAA AAAGCGACCG
 GCCATTTGTG TAGTCCAAC TAAAGTAGCC AGTCACGATT TTTCGCTGGC
 34001 AAATAGCCCG GGGGAATACA TACCCGCGAG CGTAGAGACA ACATTACAGC
 TTTATCGGGC CCCCTTATGT ATGGGCGTCC GCATCTCTGT TGTAAATGTCG
 34051 CCCCATAGGA GGTATAACAA AATTAATAGG AGAGAAAAAC ACATAAACAC
 GGGGTATCCT CCATATTGTT TTAATTATCC TCTCTTTTGT TGTATTTGTG

Figure 27A J

34151 ACATACAGCG CTTCCACAGC GGCAGCCATA ACAGTCAGCC TTACCAGTAA
TGTATGTGCG GAAGGTGTGCG CCGTCGGTAT TGTCAGTCGG AATGGTCATT

34201 AAAAGAAAAC CTATTAAAAA AACACCACTC GACACGGCAC CAGCTCAATC
TTTTCTTTTG GATAATTTTT TTGTGGTGAG CTGTGCCGTG GTCGAGTTAG

34251 AGTCACAGTG TAAAAAAGGG CCAAGTGCAG AGCGAGTATA TATAGGACTA
TCAGTGTAC ATTTTTTCCC GGTTCACGTC TCGCTCATAT ATATCCTGAT

34301 AAAAATGACG TAACGGTTAA AGTCCACAAA AAACACCCAG AAAACCGCAC
TTTTTACTGC ATTGCCAATT TCAGGTGTTT TTTGTGGGTC TTTTGGCGTG

34351 GCGAACCTAC GCCCAGAAAC GAAAGCCAAA AAACCCACAA CTTCTCAAAA
CGCTTGATG CGGGTCTTTG CTTTCGGTTT TTTGGGTGTT GAAGGAGTTT

34401 TCGTCACTTC CGTTTTCCCA CGTTACGTCA CTTCCCATTT TAAGAAAACCT
AGCAGTGAAG GCAAAAGGGT GCAATGCAGT GAAGGGTAAA ATTCTTTTGA

34451 ACAATTCCCA ACACATACAA GTTACTCCGC CCTAAAACCT ACGTCACCCG
TGTTAAGGGT TGTGTATGTT CAATGAGGCG GGATTTTGGA TGCAGTGGGC

34501 CCCC GTTCCC ACGCCCCGCG CCACGTCACA AACTCCACCC CCTCATTATC
GGGGCAAGGG TCGGGGGCGC GGTGCAGTGT TTGAGGTGGG GGAGTAATAG

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34551 ATATTGGCTT CAATCCAAAA TAAGGTATAT TATTGATGAT GTTAATTAAG
TATAACCGAA GTTAGGTTTT ATTCCATATA ATAATACTA CAATTAATTC

34601 AATTCGGATC TGCGACGCGA GGCTGGATGG CCTTCCCCAT TATGATTCTT
TTAAGCCTAG ACGCTGCGCT CCGACCTACC GGAAGGGGTA ATACTAAGAA

34651 CTCGCTTCCG GCGGCATCGG GATGCCCCGCG TTGCAGGCCA TGCTGTCCAG
GAGCGAAGGC CGCCGTAGCC CTACGGGCGC AACGTCCGGT ACGACAGGTC

34701 GCAGGTAGAT GACGACCATC AGGGACAGCT TCAAGGCCAG CAAAAGGCCA
CGTCCATCTA CTGCTGGTAG TCCCTGTCTGA AGTTCCGGTC GTTTTCCGGT

34751 GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC
CCTTGGCATT TTTCCGGCGC AACGACCGCA AAAAGGTATC CGAGGCGGGG

34801 CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCGAAACCC
GGACTGCTCG TAGTGTTTTT AGCTGCGAGT TCAGTCTCCA CCGCTTTGGG

34851 GACAGGACTA TAAAGATACC AGGCGTTTCC CCCTGGAAGC TCCCTCGTGC
CTGTCTGAT ATTTCTATGG TCCGCAAAGG GGGACCTTCG AGGGAGCACG

34901 GCTCTCCTGT TCCGACCCTG CCGCTTACCG GATACCTGTC CGCCTTTCTC
CGAGAGGACA AGGCTGGGAC GCGCAATGGC CTATGGACAG GCGGAAAGAG

34951 CCTTCGGGAA GCGTGGCGCT TTCTCATAGC TCACGCTGTA GGTATCTCAG
GGAAGCCCTT CGCACC GCGA AAGAGTATCG AGTGCACAT CCATAGAGTC

35001 TTCGGTGTAG GTCGTTGCT CCAAGCTGGG CTGTGTGCAC GAACCCCCCG
AAGCCACATC CAGCAAGCGA GGTTCGACCC GACACACGTG CTTGGGGGGC

Figure 27 AK

AAGTCGGGCT GGCGACGCGG AATAGGCCAT TGATAGCAGA ACTCAGGTTG

35101 CCGGTAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT
GGCCATTCTG TGCTGAATAG CGGTGACCGT CGTCGGTGAC CATTGTCCTA

35151 TAGCAGAGCG AGGTATGTAG GCGGTGCTAC AGAGTTCTTG AAGTGGTGGC
ATCGTCTCGC TCCATACATC CGCCACGATG TCTCAAGAAC TTCACCACCG

35201 CTAACCTACGG CTACACTAGA AGGACAGTAT TTGGTATCTG CGCTCTGCTG
GATTGATGCC GATGTGATCT TCCTGTCATA AACCATAGAC GCGAGACGAC

35251 AAGCCAGTTA CCTTCGGAAA AAGAGTTGGT AGCTCTTGAT CCGGCAAACA
TTCGGTCAAT GGAAGCCTTT TTCTCAACCA TCGAGAACTA GGCCGTTTGT

35301 AACCACCGCT GGTAGCGGTG GTTTTTTTGT TTGCAAGCAG CAGATTACGC
TTGGTGGCGA CCATCGCCAC CAAAAAACA AACGTTCTGTC GTCTAATGCG

35351 GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT
CGTCTTTTTT TCCTAGAGTT CTTCTAGGAA ACTAGAAAAG ATGCCCCAGA

35401 GACGCTCAGT GGAACGAAAA CTCACGTAA GGGATTTTGG TCATGAGATT
CTGCGAGTCA CCTTGCTTTT GAGTGCAATT CCCTAAAACC AGTACTCTAA

35451 ATCAAAAAGG ATCTTCACCT AGATCCTTTT AAATCAATCT AAAGTATATA
TAGTTTTTCC TAGAAGTGGA TCTAGGAAA TTTAGTTAGA TTTCATATAT

35501 TGAGTAACT TGGTCTGACA GTTACCAATG CTTAATCAGT GAGGCACCTA
ACTCATTTGA ACCAGACTGT CAATGGTTAC GAATTAGTCA CTCCGTGGAT

35551 TCTCAGCGAT CTGTCTATTT CGTTCATCCA TAGTTGCCTG ACTCCCCGTC
AGAGTCGCTA GACAGATAAA GCAAGTAGGT ATCAACGGAC TGAGGGGCAG

35601 GTGTAGATAA CTACGATACG GGAGGGCTTA CCATCTGGCC CCAGTGCTGC
CACATCTATT GATGCTATGC CCTCCGAAT GGTAGACCGG GGTCACGACG

35651 AATGATACCG CGAGACCCAC GCTCACCAGC TCCAGATTTA TCAGCAATAA
TTACTATGGC GCTCTGGGTG CGAGTGGCCG AGGTCTAAAT AGTCGTTATT

35701 ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA GTGGTCCTGC AACTTTATCC
TGGTCGGTCG GCCTTCCCGG CTCGCGTCTT CACCAGGACG TTGAAATAGG

35751 GCCTCCATCC AGTCTATTAA TTGTTGCCGG GAAGCTAGAG TAAGTAGTTC
CGGAGGTAGG TCAGATAATT AACAACGGCC CTTGATCTC ATTCATCAAG

35801 GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC CATTGCTACA GGCATCGTGG
CGGTCAATTA TCAAACGCGT TGCAACAACG GTAACGATGT CCGTAGCACC

35851 TGTCACGCTC GTCGTTTGGT ATGGCTTCAT TCAGCTCCGG TTCCCAACGA
ACAGTGCGAG CAGCAAACCA TACCGAAGTA AGTCGAGGCC AAGGGTTGCT

35901 TCAAGGCGAG TTACATGATC CCCCATGTTG TGCAAAAAAG CGGTTAGCTC
AGTTCCGCTC AATGTACTAG GGGGTACAAC ACGTTTTTTC GCCAATCGAG

35951 CTTCCGGTCTT CCGATCGTTG TCAGAAGTAA GTTGCCGCA GTGTTATCAC
GAAGCCAGGA GGCTAGCAAC AGTCTTCATT CAACCGCGT CACAATAGTG

Figure 2 AL

36051 AGATGCTTTT CTGTGACTGG TGAGTACTCA ACCAAGTCAT TCTGAGAATA
TCTACGAAAA GACACTGACC ACTCATGAGT TGGTTCAGTA AGACTCTTAT

36101 GTGTATGCGG CGACCGAGTT GCTCTTGCCC GCGGTCAACA CGGGATAATA
CACATACGCC GCTGGCTCAA CGAGAACGGG CCGCAGTTGT GCCCTATTAT

36151 CCGCGCCACA TAGCAGAACT TTAAAAGTGC TCATCATTGG AAAACGTTCT
GGCGCGGTGT ATCGTCTTGA AATTTTCACG AGTAGTAACC TTTTGCAAGA

36201 TCGGGGCGAA AACTCTCAAG GATCTTACCG CTGTTGAGAT CCAGTTCGAT
AGCCCCGCTT TTGAGAGTTC CTAGAATGGC GACAACCTTA GGTCAAGCTA

36251 GTAACCCACT CGTGCACCCA ACTGATCTTC AGCATCTTTT ACTTTCACCA
CATTGGGTGA GCACGTGGGT TGACTAGAAG TCGTAGAAAA TGAAAGTGGT

36301 GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC AAAATGCCGC AAAAAAGGGA
CGCAAAGACC CACTCGTTTT TGTCTTCCG TTTTACGGCG TTTTTTCCCT

36351 ATAAGGGCGA CACGGAAATG TTGAATACTC ATACTCTTCC TTTTCAATA
TATTCCCGCT GTGCCTTTAC AACTTATGAG TATGAGAAGG AAAAAGTTAT

36401 TTATTGAAGC ATTTATCAGG GTTATTGTCT CATGAGCGGA TACATATTTG
AATAACTTCG TAAATAGTCC CAATAACAGA GTACTCGCCT ATGTATAAAC

36451 AATGTATTTA GAAAAATAAA CAAATAGGGG TTCCGCGCAC ATTTCCCCGA
TTACATAAAT CTTTTTATTT GTTTATCCCC AAGGCGCGTG TAAAGGGGCT

36501 AAAGTGCCAC CTGACGTCTA AGAAACCATT ATTATCATGA CATTAAACCTA
TTTCACGGTG GACTGCAGAT TCTTTGGTAA TAATAGTACT GTAATTGGAT

36551 TAAAAATAGG CGTATCACGA GGCCCTTTCG TCTTCAAGAA TTGGATCCGA
ATTTTATCC GCATAGTGCT CCGGGAAGC AGAAGTTCTT AACCTAGGCT

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36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Figure 27AM

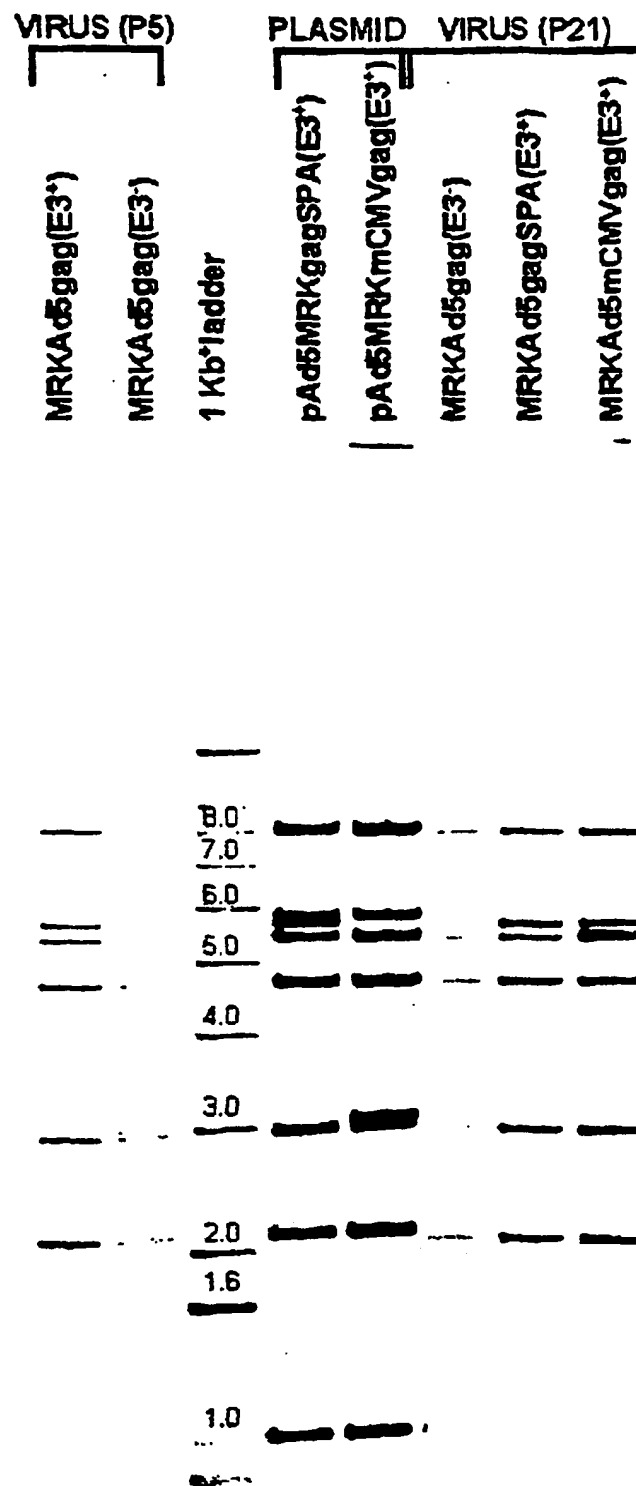


FIGURE 28

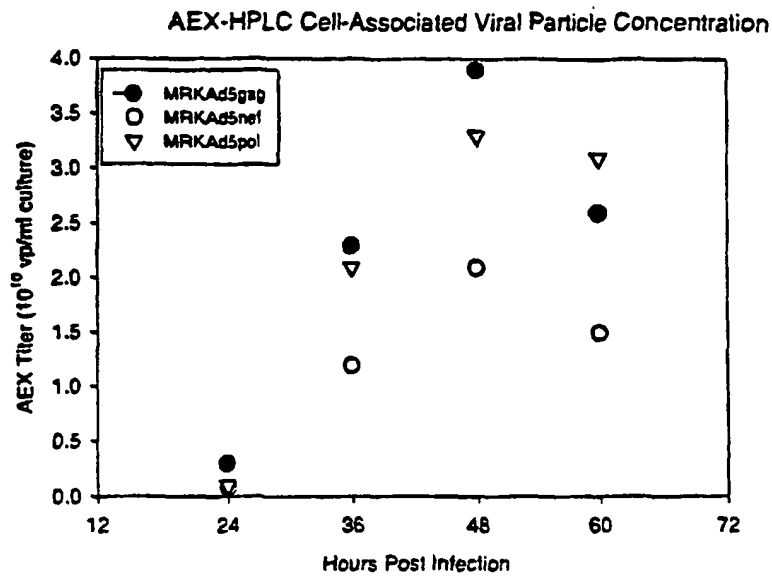


FIGURE 29A

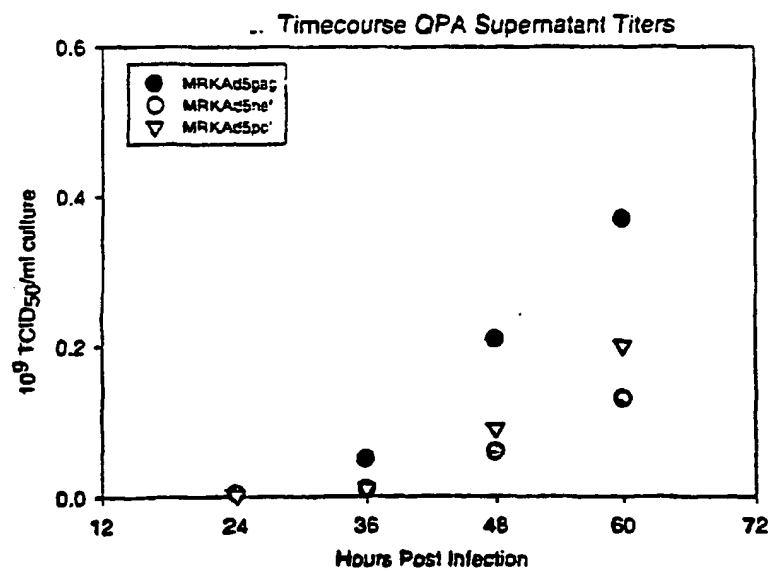


FIGURE 29B

| | |
|---|-----|
| atg gat gca atg aag aga ggg ctc tgc tgt gtg ctg ctg ctg tgt gga Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly 1 5 10 15 | 48 |
| gca gtc ttc gtt tgc ccc agc gag atc tcc att gtg tgg gcc tcc agg Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ile Val Trp Ala Ser Arg 20 25 30 | 96 |
| gag ctg gag agg ttt gct gtg aac cct ggc ctg ctg gag acc tct gag Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu 35 40 45 | 144 |
| ggg tgc agg cag atc ctg ggc cag ctc cag ccc tcc ctg caa aca ggc Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly 50 55 60 | 192 |
| tct gag gag ctg agg tcc ctg tac aac aca gtg gct acc ctg tac tgt Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys 65 70 75 80 | 240 |
| gtg cac cag aag att gat gtg aag gac acc aag gag gcc ctg gag aag Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys 85 90 95 | 288 |
| att gag gag gag cag aac aag tcc aag aag aag gcc cag cag gct gct Ile Glu Glu Glu Gln Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala 100 105 110 | 336 |
| gct ggc aca ggc aac tcc agc cag gtg tcc cag aac tac ccc att gtg Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val 115 120 125 | 384 |
| cag aac ctc cag ggc cag atg gtg cac cag gcc atc tcc ccc cgg acc Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr 130 135 140 | 432 |
| ctg aat gcc tgg gtg aag gtg gtg gag gag aag gcc ttc tcc cct gag Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu 145 150 155 160 | 480 |
| gtg atc ccc atg ttc tct gcc ctg tct gag ggt gcc acc ccc cag gac Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp 165 170 175 | 528 |
| ctg aac acc atg ctg aac aca gtg ggg ggc cat cag gct gcc atg cag Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln 180 185 190 | 576 |
| atg ctg aag gag acc atc aat gag gag gct gct gag tgg gac agg ctg Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu 195 200 205 | 624 |
| cat cct gtg cac gct ggc ccc att gcc ccc ggc cag atg agg gag ccc His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro 210 215 220 | 672 |
| agg ggc tct gac att gct ggc acc acc tcc acc ctc cag gag cag att Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile 225 230 235 240 | 720 |
| ggc tgg atg acc aac aac ccc ccc atc cct gtg ggg gaa atc tac aag Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys 245 250 255 | 768 |

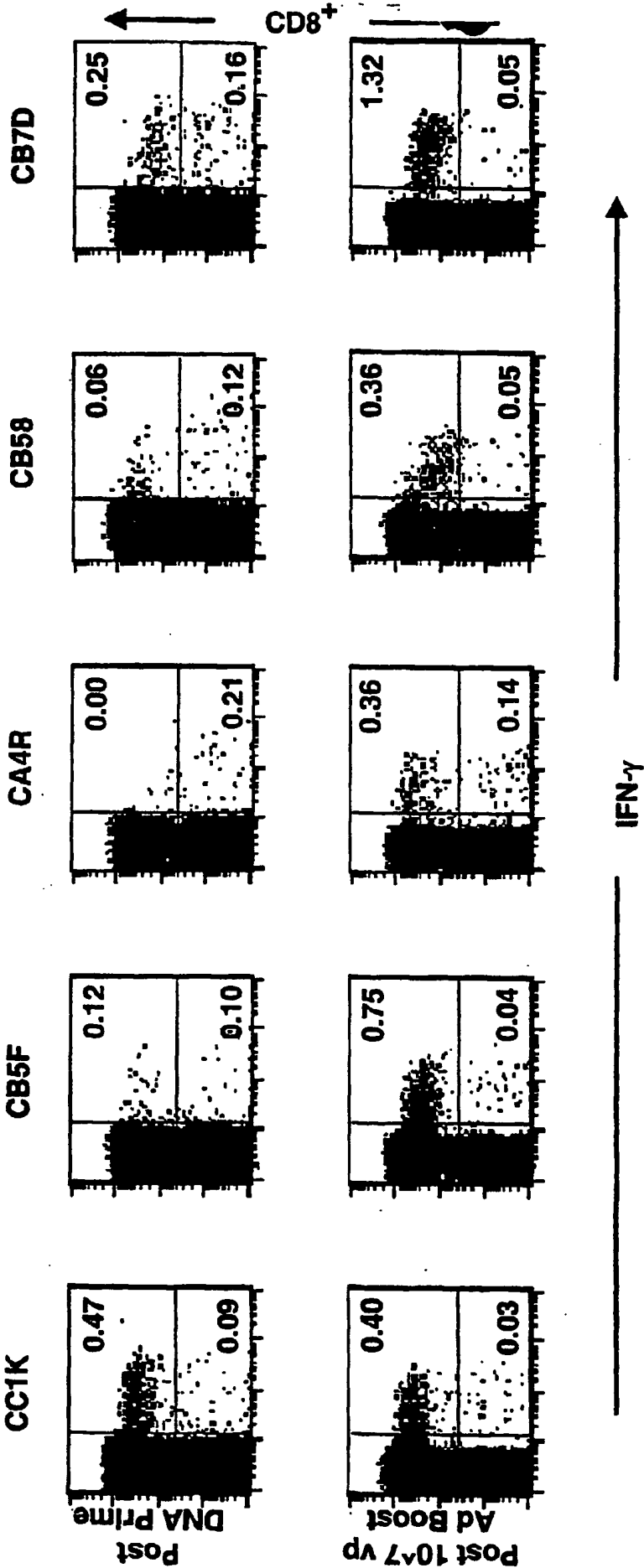
Figure 30A'

| | |
|--|------|
| agg tgg atc atc ctg ggc ctg aac aag att gtg agg atg tac tcc ccc | 816 |
| Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro | |
| 260 265 270 | |
| acc tcc atc ctg gac atc agg cag ggc ccc aag gag ccc ttc agg gac | 864 |
| Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp | |
| 275 280 285 | |
| tat gtg gac agg ttc tac aag acc ctg agg gct gag cag gcc tcc cag | 912 |
| Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln | |
| 290 295 300 | |
| gag gtg aag aac tgg atg aca gag acc ctg ctg gtg cag aat gcc aac | 960 |
| Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn | |
| 305 310 315 320 | |
| cct gac tgc aag acc atc ctg aag gcc ctg ggc cct gct gcc acc ctg | 1008 |
| Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu | |
| 325 330 335 | |
| gag gag atg atg aca gcc tgc cag ggg gtg ggg ggc cct ggt cac aag | 1056 |
| Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys | |
| 340 345 350 | |
| gcc agg gtg ctg gct gag gcc atg tcc cag gtg acc aac tcc gcc acc | 1104 |
| Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr | |
| 355 360 365 | |
| atc atg atg cag agg ggc aac ttc agg aac cag agg aag aca gtg aag | 1152 |
| Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys | |
| 370 375 380 | |
| tgc ttc aac tgt ggc aag gtg ggc cac att gcc aag aac tgt agg gcc | 1200 |
| Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala | |
| 385 390 395 400 | |
| ccc agg aag aag ggc tgc tgg aag tgt ggc aag gag ggc cac cag atg | 1248 |
| Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met | |
| 405 410 415 | |
| aag gac tgc aat gag agg cag gcc aac ttc ctg ggc aaa atc tgg ccc | 1296 |
| Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro | |
| 420 425 430 | |
| tcc cac aag ggc agg cct ggc aac ttc ctc cag tcc agg cct gag ccc | 1344 |
| Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro | |
| 435 440 445 | |
| aca gcc cct ccc gag gag tcc ttc agg ttt ggg gag gag aag acc acc | 1392 |
| Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr | |
| 450 455 460 | |
| ccc agc cag aag cag gag ccc att gac aag gag ctg tac ccc ctg gcc | 1440 |
| Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala | |
| 465 470 475 480 | |
| tcc ctg agg tcc ctg ttt ggc aac gac ccc tcc tcc cag taa (SID NO:36) 1482 | |
| Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln * (SID NO:37) | |
| 485 490 | |

Figure 30 B

Figure 31

IFN- γ Secretion against Gag 20-aa pool from CD3⁺ T cells of Monkey PBMCs



Comparison of Single-Modality Adenovirus Immunization with DNA+Adjuvant Prime/Adenovirus Boost

Immunizations

Ad Prime/Boost

DNA-CRL1005 Prime/Ad Boost

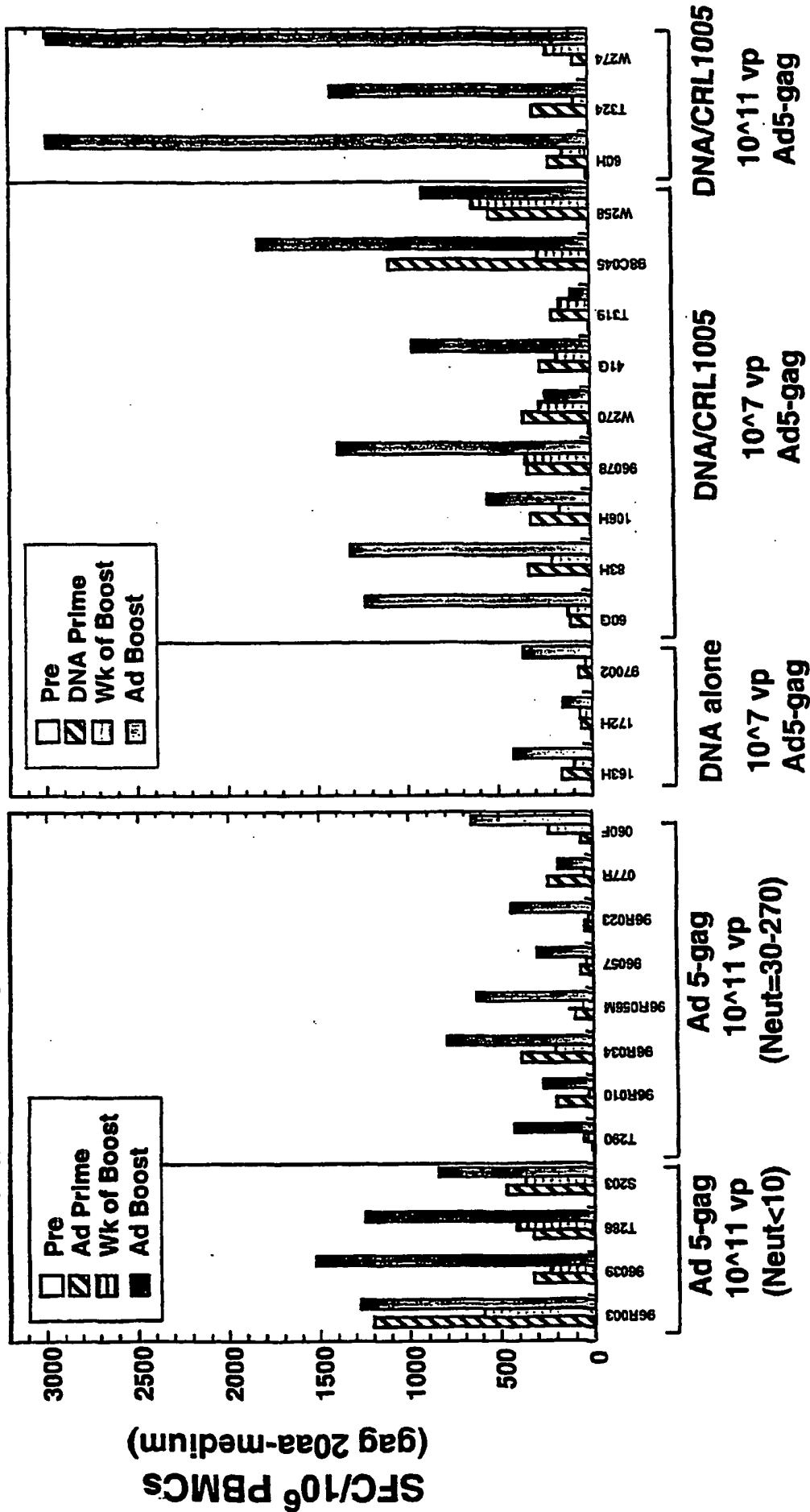


FIGURE 33A

ATGGGTGCTA GGGCTTCTGT GCTGTCTGGT GGTGAGCTGG ACAAGTGGGA GAAGATCAGG
 CTGAGGCCTG GTGGCAAGAA GAAGTACAAG CTAAAGCACA TTGTGTGGGC CTCCAGGGAG
 CTGGAGAGGT TTGCTGTGAA CCCTGGCCTG CTGGAGACCT CTGAGGGGTG CAGGCAGATC
 CTGGGCCAGC TCCAGCCCTC CCTGCAAACA GGCTCTGAGG AGCTGAGGTC CCTGTACAAC
 ACAGTGGCTA CCCTGTACTG TGTGCACCAG AAGATTGATG TGAAGGACAC CAAGGAGGCC
 CTGGAGAAGA TTGAGGAGGA GCAGAACAAG TCCAAGAAGA AGGCCAGCA GGCTGCTGCT
 GGCACAGGCA ACTCCAGCCA GGTGTCCCAG AACTACCCCA TTGTGCAGAA CCTCCAGGGC
 CAGATGGTGC ACCAGGCCAT CTCCCCCGG ACCCTGAATG CCTGGGTGAA GGTGGTGGAG
 GAGAAGGCCT TCTCCCTGA GGTGATCCCC ATGTTCTCTG CCCTGTCTGA GGGTGCCACC
 CCCCAGGACC TGAACACCAT GCTGAACACA GTGGGGGGCC ATCAGGCTGC CATGCAGATG
 CTGAAGGAGA CCATCAATGA GGAGGCTGCT GAGTGGGACA GGCTGCATCC TGTGCACGCT
 GGCCCCATTG CCCCCGGCCA GATGAGGGAG CCCAGGGGCT CTGACATTGC TGGCACCACC
 TCCACCCTCC AGGAGCAGAT TGGCTGGATG ACCAACAACC CCCCCATCCC TGTGGGGGAA
 ATCTACAAGA GGTGGATCAT CCTGGGCCTG AACAAGATTG TGAGGATGTA CTCCCCCACC
 TCCATCCTGG ACATCAGGCA GGGCCCCAAG GAGCCCTTCA GGGACTATGT GGACAGGTTC
 TACAAGACCC TGAGGGCTGA GCAGGCCTCC CAGGAGGTGA AGAACTGGAT GACAGAGACC
 CTGCTGGTGC AGAATGCCAA CCCTGACTGC AAGACCATCC TGAAGGCCCT GGGCCCTGCT
 GCCACCCTGG AGGAGATGAT GACAGCCTGC CAGGGGGTGG GGGGCCCTGG TCACAAGGCC
 AGGGTGCTGG CTGAGGCCAT GTCCCAGGTG ACCAACTCCG CCACCATCAT GATGCAGAGG
 GGCAACTTCA GGAACCAGAG GAAGACAGTG AAGTGCTTCA ACTGTGGCAA GGTGGGCCAC
 ATTGCCAAGA ACTGTAGGGC CCCCAGGAAG AAGGGCTGCT GGAAGTGTGG CAAGGAGGGC
 CACCAGATGA AGGACTGCAA TGAGAGGCAG GCCAACTTCC TGGGCAAAAT CTGGCCCTCC
 CACAAGGGCA GGCCTGGCAA CTTCTCCAG TCCAGGCCTG AGCCACAGC CCCTCCCGAG
 GAGTCCTTCA GGTTTGGGGA GGAGAAGACC ACCCCAGCC AGAAGCAGGA GCCCATTGAC
 AAGGAGCTGT ACCCCCTGGC CTCCCTGAGG TCCCTGTTTG GCAACGACCC CTCCTCCAG
 ATGGCTCCCA TCTCCCCAT TGAGACTGTG CCTGTGAAGC TGAAGCCTGG CATGGATGGC
 CCCAAGGTGA AGCAGTGGCC CCTGACTGAG GAGAAGATCA AGGCCCTGGT GGAAATCTGC
 ACTGAGATGG AGAAGGAGGG CAAAATCTCC AAGATTGGCC CCGAGAACC CTACAACACC
 CCTGTGTTTG CCATCAAGAA GAAGGACTCC ACCAAGTGA GGAAGCTGGT GGACTTCAGG
 GAGCTGAACA AGAGGACCCA GGACTTCTGG GAGGTGCAGC TGGGCATCCC CCACCCGCT
 GGCTGAAGA AGAAGAAAGT TGTGACTGTG CTGGCTGTGG GGGATGCCTA CTTCTCTGTG
 CCCCTGGATG AGGACTTCAG GAAGTACACT GCCTTCACCA TCCCTCCAT CAACAATGAG
 ACCCCTGGCA TCAGGTACCA GTACAAATGTG CTGCCCCAGG GCTGGAAGGG CTCCCCTGCC
 ATCTTCCAGT CCTCCATGAC CAAGATCCTG GAGCCCTTCA GGAAGCAGAA CCCTGACATT
 GTGATCTACC AGTACATGGC TGCCCTGTAT GTGGGCTCTG ACCTGGAGAT TGGGCAGCAC
 AGGACCAAGA TTGAGGAGCT GAGGCAGCAC CTGCTGAGGT GGGGCTGAC CACCCCTGAC
 AAGAAGCACC AGAAGGAGCC CCCCTTCCCTG TGGATGGGCT ATGAGCTGCA CCCCACAAG
 TGGACTGTGC AGCCCATTTG GCTGCCCTGAG AAGGACTCCT GGACTGTGAA TGACATCCAG
 AAGCTGGTGG GCAAGCTGAA CTGGGCCTCC CAAATCTACC CTGGCATCAA GGTGAGGCAG
 CTGTGCAAGC TGCTGAGGGG CACCAAGGCC CTGACTGAGG TGATCCCCCT GACTGAGGAG
 GCTGAGCTGG AGCTGGCTGA GAACAGGGAG ATCCTGAAGG AGCCTGTGCA TGGGGTGTAC

FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC
TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG
GGGGCCACACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG
TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG
GAGACCTGGT GGA CTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC
ACCCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTTGT GGGGGCTGAG
ACCTTCTATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG
ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC
CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC
TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG
AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC
CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG
GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC
TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC
TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT
GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT
GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC
TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC
TCCAAC TTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG
TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG
AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG
GCTGTGTTCA TCCACAACCT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG
AGGATTGTGG ACATCATTC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC
AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT
GCCAAGCTGC TGTGGAAGGG GGAGGGGGCT GTGGTGATCC AGGACAATC TGACATCAAG
GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT
GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA

SEQ ID NO: 38

FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys
 Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp
 Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser
 Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser
 Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln
 Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Gln
 Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Ala Gly Thr Gly Asn Ser Ser
 Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His
 Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys
 Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr
 Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met
 Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His
 Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser
 Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn
 Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu
 Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly
 Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala
 Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln
 Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr
 Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala
 Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met
 Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly
 Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp
 Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn
 Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln
 Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu
 Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu
 Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile
 Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys
 Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys
 Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr
 Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu
 Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu
 Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala
 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr
 Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr
 Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met
 Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln
 Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr
 Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp
 Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val
 Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro
 Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr
 Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu
 Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile
 Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu
 Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr
 Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile
 Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp
 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe
 Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile
 Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu
 Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr
 Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp
 Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile
 Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln
 Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile
 Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu
 Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn
 Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile
 Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val
 Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val
 Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro
 Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp
 Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
 Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn
 Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile
 Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val
 Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu
 Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln
 Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu
 Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln
 Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp
 Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp
 SEQ ID NO: 39

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 15/86

US CL : 435/456

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3, 235.1, 320.1, 456; 530/23.72;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---------------|---|--|
| X --- Y | WO 96/39178 (ERTL et al.) 12 December 1996 (12.12.1996), see page 5, 6, 10, 12, 13 and claims 1 and 5. | 1-3, 8-11, 18 ----- 4, 5, 13-17, 29-32, 34, 35, 37 |
| X --- Y | US 6,019,978 A (ERTL et al.) 1 February 2000, (01/02/2000), see columns 2, 7 and 8. | 1-3, 8-11, 18 ----- 4, 5, 13-17, 29-32, 34, 35, 37 |
| X,P | US 6,287,571 <i>B1</i> (ERTL et al.) 11 September 2001 (11/09/2001), see columns 2, 7, 8 and claim 1. | 1, 9, 18 |
| X --- Y | US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1997), see examples 1, 2, 25 and 26. | 1-3, 8, 9-11, 18 ----- 4,5,13-17, 29-32, 34, 35, 37 |
| Y | WANG et al. The use of an E1-deleted, replication -defective adenovirus recombinant expressing the rabies virus glycoprotein for early vaccination of mice against rabies virus. Journal of Virology (March 1997) Vol. 71, No. 5, pp 3677-3683. | 1-3, 9-11, 13-18 |

☒ Further documents are listed in the continuation of Box C.

See patent family annex.

| | |
|---|--|
| * Special categories of cited documents: | *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" document defining the general state of the art which is not considered to be of particular relevance | *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" earlier application or patent published on or after the international filing date | *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | *Z* document member of the same patent family |
| "O" document referring to an oral disclosure, use, exhibition or other means | |
| "P" document published prior to the international filing date but later than the priority date claimed | |

Date of the actual completion of the international search

06 February 2002 (06.02.2002)

Date of mailing of the international search report

19 AUG 2002

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks
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Authorized officer

Ulrike Winkler, Ph.D.

Telephone No. 703-308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| Y | NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods. | 1, 9, 29-32 |
| Y | PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficiency Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract. | 1, 9, 29-32 |
| Y | LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. | 1, 9 |
| Y | PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract. | 16 |
| Y | NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract. | 1, 9 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

| Group | Claims | |
|-------|--|---|
| 1 | 1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the <u>parallel orientation of E1</u> . In addition the vector contains a promoter and a polyadenylation signal. |
| 2 | 6, 7, 36 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> and <u>ΔE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29). |
| 3 | 12, 33 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the <u>antiparallel orientation of E1</u> . |
| 4 | 19-23, 38-42 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein. |
| 5 | 24, 27, 28, 43, 46, 47 | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle. |
| 6 | 25, 26, 44, 45 | The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in <u>addition to</u> administering a DNA plasmid vaccine. |
| 7 | 48-51, 53, 54, 56 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the <u>parallel orientation of E1</u> . |
| 8 | 48-51, 53, 54, 56 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the <u>parallel orientation of E1</u> . |
| 9 | 48-51, 53, 54, 56 | The claims are directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the <u>parallel orientation of E1</u> . |
| 10 | 52 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the <u>antiparallel orientation of E1</u> . |
| 11 | 52 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the <u>antiparallel orientation of E1</u> . |
| 12 | 52 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the <u>antiparallel orientation of E1</u> . |
| 13 | 55 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>ΔE1</u> |

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| | | and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in E1. |
| 14 | 55 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 5)</u> inserted in E1. |
| 15 | 55 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 7)</u> inserted in E1. |
| 16 | 57-61 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein. |
| 17 | 62, 65, 66 | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle. |
| 18 | 63, 64 | The claim is directed to a method of generating a cellular mediated immune response to HIV Pol protein with the recombinant adenoviral particle <u>in addition to administering a DNA plasmid vaccine.</u> |
| 19 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the parallel orientation of E1. |
| 20 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the parallel orientation of E1. |
| 21 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the parallel orientation of E1. |
| 22 | 67-70, 72, 73, 75 | The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the parallel orientation of E1. |
| 23 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in the antiparallel orientation of E1. |
| 24 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in the antiparallel orientation of E1. |
| 25 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in the antiparallel orientation of E1. |
| 26 | 71 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in the antiparallel orientation of E1. |
| 27 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 9)</u> inserted in E1. |
| 28 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 11)</u> inserted in E1. |
| 29 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type |

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| | | adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 13)</u> inserted in E1. |
| 30 | 74 | The claim is directed to an adenoviral vector that is at least partially deleted of <u>$\Delta E1$ and $\Delta E3$</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Nef protein (SEQ ID NO: 15)</u> inserted in E1. |
| 31 | 76-80 | The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein. |
| 32 | 81, 84, 85 | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. |
| 33 | 82, 83 | The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle <u>in addition to</u> administering a DNA plasmid vaccine. |
| 34 | 86a | The claim is drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from three individual vectors. |
| 35 | 86b, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from one individual vectors. |
| 36 | 86c, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-pol</i> fusion and one expressing <i>gag</i> . |
| 37 | 86d, 87, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>gag-pol</i> fusion and one expressing <i>nef</i> . |
| 38 | 86e, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from two individual vectors, one expressing <i>nef-gag</i> fusion and one expressing <i>pol</i> . |
| 39 | 86f, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> , <i>pol</i> and <i>nef</i> are expressed from a single vectors as a fusion protein. |
| 40 | 86g, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed from two individual vectors. |
| 41 | 86h, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed individually from one vector. |
| 42 | 86i, 88 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors. |
| 43 | 86j, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector. |
| 44 | 86k, 88 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector. |
| 45 | 86l, 88, 89 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed individually from one vector. |
| 46 | 86m, 88 | The claims are drawn to a multivalent vaccine wherein <i>gag</i> and <i>pol</i> are expressed as a fusion protein from one vector. |
| 47 | 86n, 88 | The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector. |
| 48 | 86o, 88 | The claims are drawn to a multivalent vaccine wherein <i>nef</i> and <i>gag</i> are expressed as a fusion protein from one vector. |

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Erdl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE

adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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